SELF-INCOMPATIBILITY IN *LOLIUM* SPECIES

by

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Abstract

Self-incompatibility in Lolium species.

A total of thirteen F_1 ryegrass families, produced by crossing genetically unrelated self-incompatible plants, were studied. These included diploid and tetraploid F_1 families which displayed a range of growth habits. The seven diploid F_1 families included annual (L. rigidum, Gaud.), biennial (L. multiflorum, L.), and perennial species (L. perenne, L.). The six tetraploid F_1 families included biennial and perennial species and, in addition, the interspecific hybrid between L. perenne and L. multiflorum. Plants in each F_1 family were crossed in a diallel array using the petri dish technique and pollen tube penetration of stigmas was observed by staining with aniline blue followed by fluorescence microscopy. Differential pollen behaviour was observed on some stigmas in all F_1 families which indicated gametophytic determination of pollen behaviour. Each F_1 family displayed a complicated pattern of compatible, incompatible and one way compatible reactions and twelve F_1 families had more than sixteen different mating types. Thus, the self-incompatibility system appeared to be controlled by more than two loci. In addition, three levels of one way compatibility were observed in some of the F_1 families. Plants which were reciprocally incompatible in both diploid and tetraploid F_1 families frequently displayed different mating types which indicated that not all the alleles in pollen and pistil had to be matched to produce an incompatible reaction.

Pollen viability was estimated by staining pollen grains with fluorescein diacetate and by culturing pollen grains *in vitro*. While pollen from all F_1 families tested could be stained only pollen from non-annual F_1 families was successfully cultured *in vitro*. A low frequency of cytological abnormalities which did not affect pollen viability, were observed in auto- and allotetraploid plants during pollen mother cell meiosis.

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Declaration

I hereby declare that this thesis has been composed by myself and that the research presented is, to the best of my knowledge, my own. Some of the work reported here has been published elsewhere. A copy of the relevant paper may be found in Appendix 2.

Signed

June, 1981.

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1. INTRODUCTION

1. INTRODUCTION

During the past thirty years forage grass cultivar improvement schemes have shown an increasing level of sophistication. At first, new varieties were developed using the technique of mass selection. More recently, the method of synthetic cultivar production has become widely established. In order to ensure complete random cross-pollination between the components of the new cultivar, the chosen individuals or lines must be high ly self-incompatible. Progress using these traditional methods, although impressive, has been slow. Recently, several novel breeding methods have been suggested which could result in a dramatic increase in productivity if fully exploited by plant breeders. These novel techniques, for example, interpopulation hybrids (Foster, 1971) and F_1 hybrids (Burton and Hart, 1964) have attractions and disadvantages. Both methods hope to capitalise on the heterosis which may occur in the F_1 generation when unrelated plants are crossed. The production of F_1 hybrids tends to be expensive and inbreeding depression may cause the loss of potentially valuable inbred lines. Interpopulation hybrids are, in some respects, less attractive than F_1 hybrids, as only 50% of the F_1 generation will be hybrid, but may still be superior to synthetic strains (Foster, 1971). As the plants used to produce an interpopulation hybrid are not inbred maintenance problems are reduced in comparison to F_1 hybrids. However, as yet, neither method has been widely adopted to produce new cultivars of forage grass species.

These novel approaches, interpopulation hybrids and F_1 hybrids, to forage grass breeding require controlled pollination between either lines or populations. Manipulation of the self-incompatibility

system of a forage grass species could give the degree of control necessary to effect a satisfactory level of cross-pollination between lines or populations. However, this requires detailed knowledge of the genetic control of the breeding system operating in a species, since this may affect the efficiency of hybrid seed production. For example, England (1974) has shown theoretically that the number of loci controlling the self-incompatibility system influences the relative proportion of hybrid seed produced in the F_1 generation. This, in turn, may reduce the yield advantage of the F_1 hybrids and hence the economic viability of this type of cultivar.

There are two main types of self-incompatibility system, gametophytic and sporophytic. The latter, found in the Cruciferae has been used extensively for the production of F_1 hybrid cultivars. Within the Gramineae self-incompatibility is widespread and has been shown to be gametophytically determined. However, knowledge of the genetic control of self-incompatibility in agriculturally important forage grasses is variable. Meadow fescue (Festuca pratensis) has been shown to have a two locus gametophytic self-incompatibility system (Lundqvist, 1955; 1961a). Cocksfoot (Dactylis glomerata) was thought to have the same type of self-incompatibility system as that found in F. pratensis, but the results were difficult to interpret due to the tetraploid nature of this species (Lundqvist, 1969b). Self-incompatibility has been observed in other species used as forage grasses including Phleum pratense and hexaploid Festuca arundinacea but has not been investigated. Studies on the genetic control of self-incompatibility in the most important temperate forage grass species, Lolium perenne and L. multiflorum have failed to reach a consensus. Therefore a study of self-incompatibility in L. perenne,

L. multiflorum and the annual L. rigidum was undertaken. In view of the importance of auto- and allotetraploid Lolium species in agriculture today, further studies of self-incompatibility included autotetraploid L. perenne and L. multiflorum and their allotetraploid hybrid.

2. LITERATURE REVIEW

2. LITERATURE REVIEW

Flowering plants exhibit a range of breeding systems, from strictly inbred to fully cross-pollinated. Whereas inbreeding is usually achieved by cleistogamy (Lactuca sativa, Triticum aestivum), outbreeding species display a variety of mechanisms, both morphogenetic and genetic, to ensure cross-fertilisation. Morphogenetic control of outcrossing separates the male and female gametes in space and/or time. In protandrous species the stamens ripen before the pistil is fully receptive (Daucus carota, Rubus idaeus), in protogynous species the pistil is receptive before the pollen is shed (Persea americana, Jugulans regia), and in monoecious species the male and female flowers are separated in space (Zea mays). Genetic diversity to facilitate outbreeding can operate by:

(i) separation of the sexes (Cannabis sativa, Humulus lupulus, Asparagus officinalis). Dioecy, the mating system of most animals is of limited importance in the angiosperms due to the high pollen requirement, inefficiency of seed production and limited range of dispersal (Allard, 1960); or by

(ii) self-incompatibility: the recognition and blockage of self or cross pollen by reason of genetic similarity. This is the major outbreeding mechanism of higher plants, with approximately fifty percent of crop plants having some kind of self-incompatibility system (Allard, 1960).

It is difficult to estimate the effectiveness of any one inbreeding or outbreeding mechanism. Certainly protandry or protogyny alone cannot completely prevent self-fertilisation, merely

hinder the process, and similarly with inbreeders cleistogamy may be variable. Table 2.1 attempts to illustrate the effectiveness of various breeding mechanisms.

Table 2.1

The effectiveness of inbreeding and outbreeding mechanisms (adapted from Frankel and Galun, 1977)

	Species	Mechanism	Natural cross pollination (%)
INBREEDERS	Lactuca sativa lettuce	partly cleistogamous	1-6
	Lycopersicon esculentum tomato	self-pollination facilitated by cone of anthers	2 *
	Pisum sativum pea	effectively cleistogamous (Allard, 1960)	<30 * (depends on cultivar)
OUTBREEDERS	Allium cepa onion	protandry (SC)	93
	Cucumis sativus cucumber	protandry, monoecy (SC)	70
	Helianthus anuus sunflower	protandry (variable SI)	20-75
	Lotus corniculatus trefoil	SI	>90
	Raphanus sativus radish	SI or SC	>85
	Trifolium spp clover	mainly SI some protandry	>90

Key: SC = self-compatible, SI = self-incompatible
* inbreeding has supplanted outbreeding (Allard, 1960).

2.1 Self-incompatibility

Over two hundred years ago Kolreuter reported that Verbascum plants did not set seed when pollinated by their own fertile pollen, but readily set seed when cross-pollinated (cited by Darwin, 1877). This phenomenon, described as self-sterility, was defined as "the incapacity of a plant to reproduce on selfing" (East and Mangelsdorf, 1925). However, Stout (1917) pointed out that self-sterility was not caused by the sterility of the male or female gametes and proposed the term self-incompatibility. There are many definitions of self-incompatibility to be found in the literature and they all emphasise the phenomenon as an outbreeding mechanism. Unfortunately, most of the definitions commonly used are restricted to the situation of self-pollination. For example, Brewbaker (1957) defined self-incompatibility as "the inability of a plant producing male and female gametes to set seed when self-pollinated". Lundqvist (1964b) stated that the phenomenon of zygote lethality should not be considered as a self-incompatible mechanism and defined self-incompatibility as "the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination". With one exception, Borago officinalis, all known self-incompatibility systems are pre-zygotic (de Nettancourt, 1977). Neither definition is entirely satisfactory as incompatibility can occur after cross-pollination when plants are genetically identical for incompatibility alleles (Stout, 1917; Frankel and Galun, 1977). Therefore, incompatibility can be defined as "the inability of plants having functional gametes to set seeds when either self-pollinated or cross-pollinated by genetically related individuals" (based on Frankel and Galun, 1977).

The phenomenon of self-incompatibility can be categorised into gametophytic and sporophytic on the basis of the genetical control of pollen behaviour. In the sporophytic self-incompatibility system the incompatible phenotype of the pollen is determined by the genotype of the pollen producing plant. In contrast, the individual microspore determines the phenotype of the pollen in gametophytic self-incompatibility systems. The fundamental difference between the two systems is thought to be the time at which the S-alleles act to produce the incompatibility substances or precursors (Pandey, 1958; 1960). Pandey (1958; 1960), suggested that with sporophytic control the time of action of the S-alleles must be after anaphase II in pollen mother cell meiosis and before the separation of the four microspores from the common cytoplasm; where pollen behaviour is determined gametophytically the time of S-allele action must be after cytokinesis.

At anthesis pollen grains are either binucleate or trinucleate. The number of nuclei in mature pollen grains was constant in a genus and sometimes within a whole family (Brewbaker, 1957; 1959). By combining Lewis' (1956) observations on the site of inhibition of incompatible pollen tubes and his own observations of pollen cytology, Brewbaker (1957) formulated the following generalisations:

(i) species having sporophytic self-incompatibility systems have trinucleate pollen grains at anthesis and incompatible pollen tubes are inhibited on the stigma;

and

(ii) species having gametophytic self-incompatibility systems have binucleate pollen grains at anthesis and incompatible pollen tubes are inhibited in the style.

There are, however, exceptions to these relationships. For example, the Gramineae combine gametophytic self-incompatibility with trinucleate pollen and stigma inhibition, and Oenothera species combine gametophytic self-incompatibility with binucleate pollen and stigmatic inhibition.

2.1.1 Sporophytic self-incompatibility

In some species differences in floral morphology are associated with sporophytic incompatibility in order to reinforce the mechanism of pollen rejection. Incompatibility systems characterised by such features are described as heteromorphic. Species in which sporophytic incompatibility is not associated with differences in floral morphology are described as homomorphic.

Heteromorphic sporophytic self-incompatibility has been reported in several ornamental families (Linaceae, Lythraceae, Oxalidaceae) but is rare in economic crops (de Nettancourt, 1977).

Homomorphic sporophytic self-incompatibility systems have been reported for numerous species, many of which are of great economic importance (principally the Cruciferae, Compositae and Convulvulaceae) (Frankel and Galun, 1977).

2.1.1.1 Heteromorphic sporophytic self-incompatibility.

Floral differences appear in the style length and anther level with species being either distylic (Primula spp.) or tristylic (Lythrum salicaria).

In Primula vulgaris the two types of flowers are referred to as pin (long style, short anther filaments) and thrum (short style, long anther filaments). Compatible pollinations could only occur between pollen grains from anthers which were at the same height in

the flower as the stigma (de Nettancourt, 1977; Lewis, 1979). The system was found to be controlled by one gene with two alleles S and s. Thrum plants were found to be heterozygous Ss and pin plants were homozygous recessive. As there was sporophytic determination of pollen behaviour all thrum pollen behaved as 'S'. Thus, the population was maintained with equal proportions of pin and thrum flowers (Frankel and Galun, 1977).

In tristylic species (Lythrum, Oxalis) the flowers had long, medium and short styles, and each flower had two sets of anthers at levels which did not correspond to the level of the stigma. Again, compatible pollinations only occurred between pollen from anthers and stigmas at the same height. The genetic control of tristyly was found to be based on two genes (S and M) with epistatic relations (Frankel and Galun, 1977).

2.1.1.2 Homomorphic sporophytic self-incompatibility.

Sporophytic control of self-incompatibility was first described in 1912 for Cardamine pratensis by Correns (cited in de Nettancourt, 1977) and later in Capsella by Riley (1936). In these species self-incompatibility was thought to be genetically controlled by two diallelic loci (de Nettancourt, 1977). In addition, Verma et al. (1977) and Lewis (1977) have recently postulated that the sporophytic self-incompatibility system in Eruca sativa is controlled by three multiallelic loci.

However, the majority of species having homomorphic self-incompatibility systems with sporophytic determination of pollen behaviour are usually controlled by a single multiallelic locus (de Nettancourt, 1972). This type of incompatibility system was

first described independently by Hughes and Babcock (1950) working with Crepis foetida subsp. rhoeadifolia, and Gerstel (1950) working with Parthenium argentatum (both members of the Compositae family). They postulated:

(i) one gene with multiple alleles;

(ii) sporophytic control of pollen behaviour; and

(iii) dominance of alleles in the pollen and independent action of alleles in the pistil.

It is now known that dominance of S-alleles can occur in the pistil and independent action of the S-alleles can occur in the pollen, and that independence/dominance relationships do not always differ in pollen and pistil (de Nettancourt, 1977). Also, the classification of alleles in a linear order of dominance may vary from anther to pistil.

2.1.2 Gametophytic self-incompatibility

All gametophytic self-incompatibility systems are thought to be homomorphic (de Nettancourt, 1977; Frankel and Galun, 1977), and are widespread in crop plants occurring in the families Leguminosae, Gramineae, Solanaceae, Rosaceae, as well as in several ornamental species (Oenothera, Lilium, Antirrhinum, Nemesia) (Frankel and Galun, 1977). In self-incompatible species in which pollen behaviour is determined gametophytically, a pollen grain can germinate, grow and achieve fertilisation provided that the S-allele it carries is not present in the diploid tissue of the female organs (de Nettancourt, 1972).

Although the phenomenon of self-sterility or self-incompatibility had been known since before Darwin, it was not until 1925 that East and Manglesdorf (1925) working with Nicotiana, provided a genetic

interpretation of the system. They postulated:

(i) one gene with many alleles;

(ii) gametophytic determination of pollen behaviour; and

(iii) independent action of alleles in the style.

Arasu (1968) pointed out that the same conclusions were reached by Prell (1921), Lehmann (1926) and Filzer (1926) (all cited by Arasu, 1968). However, it was the terminology used by East and Manglesdorf (1925) to describe the self-incompatibility alleles (S_1, S_2, S_3 ..... S_n) which became widely adopted and the papers of Prell (1921), Lehmann (1926) and Filzer (1926) are not often quoted.

The gametophytic determination of pollen behaviour results in three possible types of pollination (Fig. 2.1.b):

(i) fully compatible, between plants with no alleles in common;

(ii) half compatible, between plants with one allele in common; and

(iii) incompatible, between plants with both alleles in common.

In contrast, in the sporophytic system, pollen from a single plant arriving on a stigma is either all compatible or all incompatible (Fig. 2.1.a).

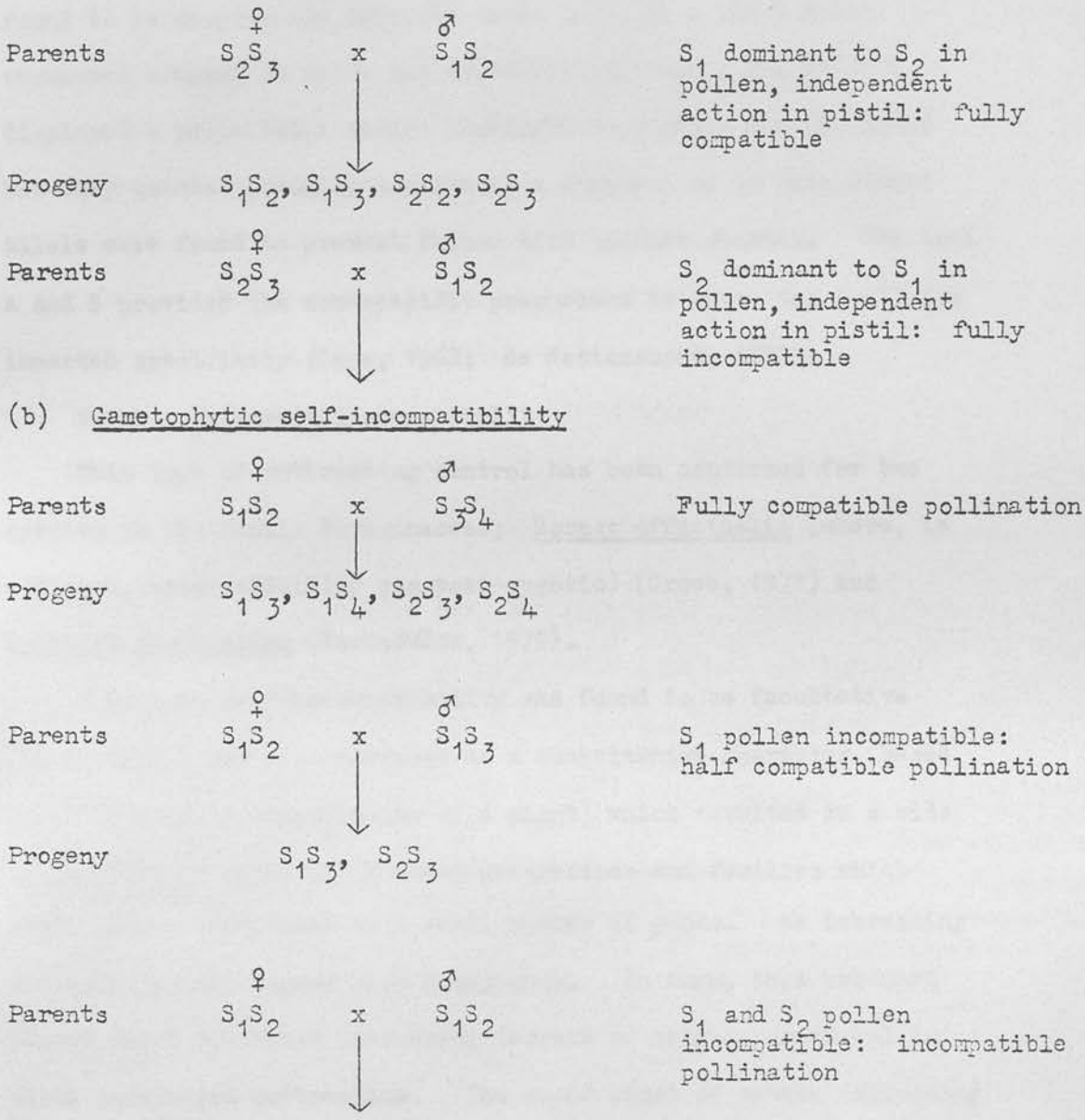
2.1.3 Gametophytic - Sporophytic systems

The self-incompatibility system of Theobroma cacao provides the only known example of a mechanism combining, during microsporogenesis, both gametophytic and sporophytic control of the incompatibility phenotype of the pollen. Incompatible pollen germinated and grew normally, fertilisation occurred and after a few nuclear divisions the embryo and endosperm aborted (Knight and Rogers, 1955). In

Fig. 2.1

Types of pollination in sporophytic and gametophytic self-incompatibility systems

(a) Sporophytic self-incompatibility



(b) Gametophytic self-incompatibility

contrast to other self-incompatibility systems, incompatibility in T. cacao was extremely wasteful of female gametes (Cope, 1976).

The genetic control of the self-incompatibility system was found to be complex and involved three loci, S, A and B which regulated syngamy on self- and cross-pollination. The S-locus displayed a polyallelic series showing independence and dominance but only gametes actually containing a dominant or an independent allele were found to prevent fusion with similar gametes. The loci A and B provided the non-specific precursors to which the S-alleles imparted specificity (Cope, 1962; de Nettancourt, 1977).

2.1.4 Polygenic incompatibility

This type of outbreeding control has been confirmed for two species in the family Boraginaceae; Borago officinalis (where, in addition, incompatibility was post-zygotic) (Crowe, 1971) and Myosotis scorpioides (Varopoulos, 1979).

Polygenic self-incompatibility was found to be facultative and versatile and was expressed as a quantitative character (based on the degree of homozygosity of a plant) which resulted in a wide and continuous variation between generations and families which could not be attributed to a small number of genes. As inbreeding proceeded plants became more homozygous. In turn, this produced plants which exhibited increasing degrees of self-incompatibility which encouraged outbreeding. The rapid onset of severe inbreeding depression indicated that the apparently weak outbreeding system was sufficient to prevent widespread self-fertilisation in nature (Crowe, 1971).

De Nettancourt (1977) suggested that polygenic restrictions

to inbreeding are probably more widespread in nature than is appreciated. Varopoulos (1979) considered that results obtained for Medicago spp. (Fyfe, 1957) and for certain genotypes of Vicia faba (Drayner, 1959) indicated polygenic control of the self-incompatibility system.

2.1.5 Differences between sporophytic and gametophytic self-incompatibility systems

Many attempts have been made to classify sporophytic and gametophytic self-incompatibility systems. Categories commonly used include: the uniformity of the pollen reaction on the stigma; the occurrence of one way compatibility; homozygosity of S-alleles; effect of polyploidy on the system; site of pollen tube inhibition in incompatible reactions; and pollen cytology (Table 2.2).

2.1.6 Complex systems: multilocus gametophytic self-incompatibility systems

A range of complexity in the genetical control of gametophytic self-incompatibility systems has been found from the one locus system already described (Section 2.1.2) to the four locus system found in Beta vulgaris (Section 2.1.6.4).

2.1.6.1 Solanaceae

In the Solanaceae, outbreeding was usually found to be promoted by a one locus gametophytic self-incompatibility system (Abdalla and Hermsen, 1971). However, two locus control of incompatibility has been demonstrated for Physalis ixocarpa (Pandey, 1957), Solanum pinnatisectum (Pandey, 1962a), S. phureja and S. stenotomum (Abdalla and Hermsen, 1971). These systems were thought to have arisen from a duplication of the S-locus following the induction of polyploidy. Self-incompatibility was controlled by two independently segregating

Table 2.2

Differences between sporophytic and gametophytic self-incompatibility systems

	Characteristic	Sporophytic	Gametophytic
(1)	pollen reaction on stigmatic surface	uniform pollen behaviour	differential pollen behaviour
(2)	one way compatibility	occurs due to dominance of S-alleles in pollen	does not occur in simple systems
(3)	individuals homozygous for S-alleles	individuals homozygous for S-alleles are a normal part of the system	individuals homozygous for S-alleles are precluded by the system
(4)	induced polyploidy	does not affect the functioning of the system	results in breakdown of self-incompatibility and self-fertilisation occurs
(5)	site of inhibition of incompatible pollen tubes (generalisations)	stigmatic inhibition	stylar inhibition
(6)	pollen cytology (generalisations)	trinucleate pollen	binucleate pollen

loci, S and R, with multiple alleles. In Physalis, identity at either locus in pollen and pistil was sufficient to cause an incompatible reaction. In addition, there was either independent action or epistasis between the alleles of the two loci (Pandey, 1957). This resulted in a low level of cross-compatibility between related individuals. In the three Solanum species, the genetic control of incompatibility was found to be more complex than in P. ixocarpa. In S. pinnatisectum, incompatibility depended upon dominance relationships between the S-alleles and epistatic interactions between the alleles of the S and R loci (Pandey, 1962a). Although the incompatibility system in S. phureja and S. stenotomum was found to be similar to that in S. pinnatisectum (epistasis occurred between S and R alleles), dominance relationships between S or R alleles have not been detected (Abdalla and Hermsen, 1971).

2.1.6.2 Gramineae: two locus systems

During the last quarter-century many species within the family Gramineae have been investigated and the majority have been shown to possess a self-incompatibility system genetically controlled by two loci, with gametophytic determination of pollen behaviour.

The system was first postulated to be operating in rye (Secale cereale) by Lundqvist (1954). The two loci, termed S and Z, were found to be independent and both had multiple alleles. Identity between pollen and pistil at either one of the two loci alone did not produce an incompatible reaction. Each specific pair of S and Z alleles led to one unique specificity and identity between specificities in pollen and pistil produced an incompatible reaction. Thus, it appeared that the two loci behaved as a single physiological

unit, with the alleles co-operating to produce the incompatibility specificity (Lundqvist, 1956). A similar system of genetic control of self-incompatibility was demonstrated for Festuca pratensis (Lundqvist, 1955; 1961a) and Phalaris coerulescens (Hayman, 1956), and was assumed to be widespread within the Gramineae. Hayman (1956) and Lundqvist (1954) reached the same conclusions independently by the use of different experimental designs.

Although dealing with self-incompatible plants, Lundqvist (1954) found it possible to self S. cereale under certain conditions. Progeny produced as a result of selfing were crossed to each other in all possible combinations. When the self-incompatibility system is controlled genetically by one locus with gametophytic determination of pollen behaviour only three genotypes would be expected in the progeny resulting from selfing if the parent plant was heterozygous (Fig. 2.2), and more than three genotypes were observed in the F_1 families studied by Lundqvist (1954; 1955). If, however, the system was controlled by two loci, with gametophytic determination of pollen behaviour, there would be nine possible mating types in the F_1 progeny (if the parent plant was heterozygous at both loci) (Fig. 2.3).

Within the nine mating types there were three groups of plants (Lundqvist, 1954; 1955):

(i) both loci heterozygous, $S_1S_2Z_3Z_4$;

(ii) one locus heterozygous one locus homozygous, for example $S_1S_2Z_3Z_3$; and

(iii) both loci homozygous, for example $S_1S_1Z_3Z_3$.

This, in turn, resulted in three levels of one way compatibility corresponding to the three possible degrees of heterozygosity (Fig. 2.3). The plant $S_1S_2Z_3Z_4$ (double heterozygote) was compatible as a

Fig. 2.2

Gametophytic self-incompatibility system: Selfing a plant with one heterozygous locus

Parent S_1S_2

↓

Progeny S_1S_1, S_1S_2, S_2S_2 3 genotypes

ratio 1 : 2 : 1

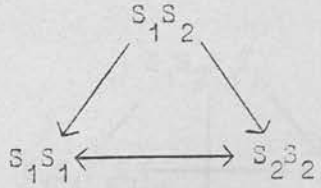
crossing progeny in all possible combinations

♀ \ ♂	S_1S_1	S_2S_2	S_1S_2
S_1S_1	-	+	+
S_2S_2	+	-	+
S_1S_2	-	-	-

Key

\+ compatible
\- incompatible

one way compatibility (2 levels)



Key

cross is compatible only in direction arrow points

Fig. 2.3

Gametophytic self-incompatibility system: Selfing a plant with two heterozygous loci

Parent $S_1S_2Z_3Z_4$

↓

Progeny $S_1S_2Z_3Z_4$, $S_1S_1Z_3Z_4$, $S_2S_2Z_3Z_4$, $S_1S_2Z_3Z_3$, $S_1S_2Z_4Z_4$

ratio 4 : 2 : 2 : 2 : 2

Progeny $S_1S_1Z_3Z_3$, $S_1S_1Z_4Z_4$, $S_2S_2Z_3Z_3$, $S_2S_2Z_4Z_4$

ratio 1 : 1 : 1 : 1

9 genotypes

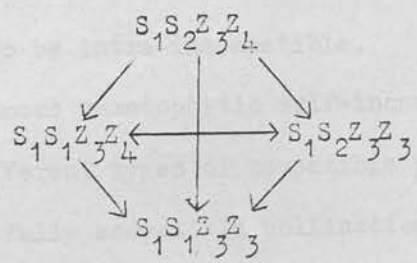
Crossing progeny in all possible combinations

♀ \ ♂	$S_1S_2Z_3Z_4$	$S_1S_1Z_3Z_4$	$S_2S_2Z_3Z_4$	$S_1S_2Z_3Z_3$	$S_1S_2Z_4Z_4$	$S_1S_1Z_3Z_3$	$S_1S_1Z_4Z_4$	$S_2S_2Z_3Z_3$	$S_2S_2Z_4Z_4$
$S_1S_2Z_3Z_4$	-	-	-	-	-	-	-	-	-
$S_1S_1Z_3Z_4$	+	-	+	+	+	-	-	+	+
$S_2S_2Z_3Z_4$	+	+	-	+	+	+	+	-	-
$S_1S_2Z_3Z_3$	+	+	+	-	+	-	+	-	+
$S_1S_2Z_4Z_4$	+	+	+	+	-	+	-	+	-
$S_1S_1Z_3Z_3$	+	+	+	+	+	-	+	+	+
$S_1S_1Z_4Z_4$	+	+	+	+	+	+	-	+	+
$S_2S_2Z_3Z_3$	+	+	+	+	+	+	+	-	+
$S_2S_2Z_4Z_4$	+	+	+	+	+	+	+	+	-

Key

\+ compatible
\- incompatible

One way compatibility (3 levels)



Key

cross is compatible only in direction arrow points

male with all genotypes but incompatible as a female with all males. A plant heterozygous at one locus only, $S_1S_2Z_3Z_3$, when used as a male was incompatible with the double heterozygote but compatible with all other genotypes. A plant which was doubly homozygous, $S_1S_1Z_3Z_3$, was incompatible as a male with the double heterozygote and some genotypes heterozygous at one locus (Lundqvist, 1955) (Fig. 2.3). Pollen with one allele matched in the style was able to function without any disadvantage when compared to pollen with no alleles matched in the style (Lundqvist, 1956; 1958).

Hayman (1956) crossed two inter-fertile plants and then crossed the resulting progeny in a diallel array. If one locus controlled the self-incompatibility system with gametophytic determination of pollen behaviour then only four genotypes are expected in the F_1 if the two parents had no alleles in common (Fig. 2.4.a). If two loci controlled the gametophytic self-incompatibility system, then a maximum of sixteen mating types would be expected in the F_1 progeny if the parents had no alleles in common (Fig. 2.4.b). Hayman (1956) found more than four and less than sixteen mating types in the F_1 progenies he examined and proposed that this could be explained if the parents were assumed to have alleles in common. This assumption also explained the observation of one way compatibility within the F_1 progenies (Fig. 2.5). Groups of plants which has the same genotype were found to be intra-incompatible.

A two locus gametophytic self-incompatibility system results in three different types of compatible pollination:

(i) fully compatible pollinations between individuals with no alleles, or one allele, in common ($S_1S_2Z_1Z_2$ ♀ x $S_3S_4Z_3Z_4$ ♂) (Fig. 2.4.b);

Fig. 2.4

Gametophytic self-incompatibility system

(a) two plants with one heterozygous locus each crossed

	♀		♂	
Parents	S_1S_2	x	S_3S_4	
	↓			
Progeny	S_1S_3,	S_1S_4,	S_2S_3,	S_2S_4 4 genotypes
ratio	1 :	1 :	1 :	1

All progeny are compatible with each other when crossed in all possible combinations

(b) two plants with two heterozygous loci each crossed

	♀		♂	
Parents	$S_1S_2Z_1Z_2$	x	$S_3S_4Z_3Z_4$	
	↓			
Progeny	$S_1S_3Z_1Z_3$	$S_1S_4Z_1Z_3$	$S_2S_3Z_1Z_3$	$S_2S_4Z_1Z_3$
	$S_1S_3Z_1Z_4$	$S_1S_4Z_1Z_4$	$S_2S_3Z_1Z_4$	$S_2S_4Z_1Z_4$
	$S_1S_3Z_2Z_3$	$S_1S_4Z_2Z_3$	$S_2S_3Z_2Z_3$	$S_2S_4Z_2Z_3$
	$S_1S_3Z_2Z_4$	$S_1S_4Z_2Z_4$	$S_2S_3Z_2Z_4$	$S_2S_4Z_2Z_4$

All sixteen genotypes produce a unique mating type when crossed in all possible combinations (all cross-compatible)

Fig. 2.5

Gametophytic self-incompatibility system: crossing two plants having two heterozygous loci where alleles are common

	♀		♂
Parents	$S_1S_2Z_1Z_2$	x	$S_1S_3Z_1Z_4$
		↓	(pollen type S_1Z_1 incompatible)
Progeny	$S_1S_1Z_1Z_4$	$S_1S_3Z_1Z_1$	$S_2S_3Z_1Z_1$
	$S_1S_1Z_2Z_4$	$S_1S_3Z_1Z_2$	$S_2S_3Z_1Z_2$
	$S_1S_2Z_1Z_4$	$S_1S_3Z_1Z_4$	$S_2S_3Z_1Z_4$
	$S_1S_2Z_2Z_4$	$S_1S_3Z_2Z_4$	$S_2S_3Z_2Z_4$

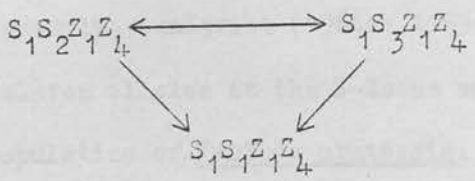
12 genotypes

Progeny crossed in all possible combinations

♀ \ ♂	$S_1S_1Z_1Z_4$	$S_1S_1Z_2Z_4$	$S_1S_2Z_1Z_4$	$S_1S_2Z_2Z_4$	$S_1S_3Z_1Z_1$	$S_1S_3Z_1Z_2$	$S_1S_3Z_1Z_4$	$S_1S_3Z_2Z_4$	$S_2S_3Z_1Z_1$	$S_2S_3Z_1Z_2$	$S_2S_3Z_1Z_4$	$S_2S_3Z_2Z_4$
$S_1S_1Z_1Z_4$	-	+	+	+	+	+	+	+	+	+	+	+
$S_1S_1Z_2Z_4$	+	-	+	+	+	+	+	+	+	+	+	+
$S_1S_2Z_1Z_4$	-	+	-	+	+	+	+	+	+	+	+	+
$S_1S_2Z_2Z_4$	+	-	+	-	+	+	+	+	+	+	+	+
$S_1S_3Z_1Z_1$	+	+	+	+	-	+	+	+	+	+	+	+
$S_1S_3Z_1Z_2$	+	+	+	+	-	-	+	+	+	+	+	+
$S_1S_3Z_1Z_4$	-	+	+	+	-	+	-	+	+	+	+	+
$S_1S_3Z_2Z_4$	+	-	+	+	+	+	+	-	+	+	+	+
$S_2S_3Z_1Z_1$	+	+	+	+	+	+	+	+	-	+	+	+
$S_2S_3Z_1Z_2$	+	+	+	+	+	+	+	+	-	-	+	+
$S_2S_3Z_1Z_4$	+	+	+	+	+	+	+	+	-	+	-	+
$S_2S_3Z_2Z_4$	+	+	+	+	+	+	+	+	+	+	+	-

Key
+ compatible
- incompatible

One way compatibility (2 levels)



Key
cross is compatible only in direction arrow points

(ii) pollinations in which only three-quarters of the pollen grains can achieve fertilisation (at least two alleles must be common to the parents, for example $S_1S_2Z_1Z_2$ ♀ x $S_1S_3Z_1Z_4$ ♂) (Fig. 2.5); and

(iii) half compatible pollinations, when only half the pollen grains can achieve fertilisation (at least three alleles common between parents, for example $S_1S_2Z_1Z_2$ ♀ x $S_1S_2Z_1Z_3$ ♂).

Differential pollen behaviour on stigmas was observed by Lundqvist (1954; 1955) and by Hayman (1956) who classified the pollinations he observed as incompatible, half compatible, three-quarters compatible and fully compatible. However, pollinations between plants of known genotypes often produced less than the maximum percentage of compatible grains expected (Lundqvist, 1961a).

Other species in which the two locus gametophytic self-incompatibility system has been demonstrated include Hordeum bulbosum (Lundqvist, 1962a) Dactylis ascherscniana (Lundqvist, 1965), Briza media (Murray, 1974) and more recently Lolium perenne (Cornish, Hayward and Lawrence, 1979a).

The breeding efficiency (the probability for any two plants being cross-compatible) of species having a two locus system is superior to those species where self-incompatibility is controlled genetically by a single locus as the number of specificities within a given population will, in theory, correspond to the product of the number of alleles segregating at each of the two loci (Lundqvist, 1964a). For example, Lundqvist (1964a; 1969a) estimated that there were at least eleven alleles at the S-locus and twelve alleles at the Z-locus in a population of Festuca pratensis. Only in one species

investigated (Dactylis aschersoniana) has there been any indication of a limited number of S and Z alleles (Lundqvist, 1965). The experiments with D. aschersoniana involved ten plants collected from an isolated population, which were selfed. The progeny from four of these selfed plants were analysed in diallel crosses. In two of these families only one locus was found to be segregating and results obtained were explained on the basis of only two alleles at the segregating locus. All the plants must have been homozygous for the same allele at the other locus, in each family. Therefore, a maximum of three alleles were present in these two families. In the other two families analysed, seven and four mating types were found thus indicating the involvement of two incompatibility loci, and more than three alleles.

The efficiency of the two locus self-incompatibility system would be reduced if the two loci, S and Z, were found to be linked. No evidence of linkage has been obtained in a number of studies (Hayman, 1956; Lundqvist, 1961a; Cornish et al., 1979b).

Studies of incompatibility in induced tetraploids of Secale cereale (Lundqvist, 1957) and Festuca pratensis (Lundqvist, 1962b) demonstrated that there was no breakdown of the system, which commonly occurred in polyploids of species having a one locus gametophytic self-incompatibility system. However, the system in these tetraploids was modified such that one allele from each locus (S and Z) of the diploid pollen when matched in the pistil was sufficient to produce an incompatible reaction. Thus, non-identical S-genotypes were found to be reciprocally incompatible and in turn, this produced a disturbed segregation pattern with the possibility of more than sixteen

S-genotypes and no distinct incompatibility groups (Lundqvist, 1957; 1962b; Østerbye et al., 1980).

Progenies produced as a result of selfing plants of Dactylis glomerata (a natural, self-incompatible, autotetraploid species) were intercrossed in all possible combinations (Lundqvist, 1969b). As tetrasomic inheritance is complex, the analysis was restricted to mean proportions of compatible pollen observed. The results obtained gave a much higher level of cross-compatibility between sibs than the theoretical expectations for a tetraploid. Self-fertility (Sf) alleles, and loss of activity of the self-incompatibility gene were postulated to account for this discrepancy (Lundqvist, 1969b). The proposed Sf alleles would reduce the number of S-Z specificities in the pollen and pistil and therefore result in an increase in cross-compatibility. Despite the presence of Sf alleles, some S-alleles remained functional and prevented self-fertilisation (Lundqvist, 1969b).

2.1.6.3 Gramineae: multilocus systems

Although the two locus system has been reported for the majority of grasses investigated, recent work indicates that the two locus gametophytic self-incompatibility system may not be universal within the Gramineae (Østerbye et al., 1980). Two genera, Briza (Murray, 1979), and Lolium (Hayward and Wright, 1971; Spoor, 1976; Hay, 1978 (see Appendix 2)) contain species having gametophytic self-incompatibility systems which appear to be controlled by more than two loci. Evidence of more than one type of incompatibility system operating within a family has been found in the Solanaceae (Pandey, 1962a; Abdalla and Hermsen, 1971), Boraginaceae (Crowe, 1971), and

Caryophyllaceae (Lundqvist, 1979).

Lolium perenne was first reported (Weimarck, 1968) to possess the standard two locus control of self-incompatibility with gametophytic pollen determination. However, the evidence presented, based on percentage seed set, consists of the results of matings between three clones which appear to have different mating types. A system of one locus control could produce a maximum of four mating types, and the claim for a two locus system based on differential pollen behaviour does not seem justified (Hayward and Wright, 1971; Spoor, 1976).

Not convinced by Weimarck's (1968) data, Hayward and Wright (1971) investigated incompatibility relationships between full sibs in three F_1 families of Lolium perenne. They used Lundqvist's (1961b) petri dish technique and stained pollinated pistils with cotton blue. Pollen grains were scored as compatible (when not stained) and incompatible (when darkly stained) and results were presented as proportions of compatible grains observed. However, there was some confusion over non-viable pollen grains which remained unstained in cotton blue. The percentages of emptied grains were expected to be 0%, 50%, 75% and 100%, which corresponds to an incompatible pollination and the three types of compatible pollination expected on a two locus gametophytic self-incompatibility system. Hayward and Wright (1971) found that percentages of emptied pollen grains did not fall into these classes, even after allowing for the presence of non-viable (non-stained) pollen grains. Within each family investigated by Hayward and Wright (1971) each plant had a unique mating type (maximum of fourteen) but the results could not be fitted into a one or the two locus system as postulated by Lundqvist (1954; 1955). Gametophytic control was indicated by:

(i) differential pollen behaviour on the stigma;

(ii) the high proportion of cross-compatible matings (which was higher than expected on a sporophytic system); and

(iii) the occurrence of one way compatibility.

Spoor (1976) also studied the incompatibility relationships in a family of twenty-eight full sibs of L. perenne, but in an attempt to overcome the difficulties caused by the staining technique used by Hayward and Wright (1971), located pollen tubes growing through the stigma by means of the callose fluorescence reaction (Lalouette, 1967). Each plant had a unique mating type, with a complicated pattern of cross-compatibility, cross-incompatibility and reciprocal differences in compatibility. Differential pollen behaviour on stigmas indicated gametophytic determination of pollen behaviour and reciprocal differences were suggested to be the result of the parents having alleles in common. Since a maximum of sixteen mating types would be expected in an F_1 progeny from two parents with no alleles in common, a novel system with three loci was postulated to be controlling the self-incompatibility system in L. perenne (Spoor, 1976).

The most recent study of the genetics of self-incompatibility in L. perenne was presented by Cornish et al. (1979a). Seven families of full sibs (thirty to forty plants per family), which were the products of wide crosses between unrelated plants, were investigated. In each family, six to eight plants were chosen at random as pollinators, and crossed to every other member of the family as well as to each other. Each pollinator was shown to have a different mating type and genotypes were then provisionally assigned to the original parents of the cross and to each pollinator. The results of the crosses between the pollinators and the remaining plants of

the family were then used to classify the latter. Further crosses were performed within intra-incompatible groups to confirm the initial classification. In no case was a diallel completed; plants were assigned to genotypes on the basis of their reactions with the chosen pollinators and cases of one way compatibility between plants were only found in two of the families analysed. Only four types of pollination were recognised: fully incompatible, half compatible, three-quarters compatible and fully compatible. This classification effectively precluded any alternative explanation of the results. The percentage of compatible grains was estimated visually and pollen tube growth was followed by staining with aniline blue and fluorescence microscopy, according to the technique of Martin (1959). The four types of pollination and the fact that a maximum of fourteen mating types were found was taken as evidence for a two locus gametophytic self-incompatibility system operating in this species (Cornish et al., 1979a).

On this basis, Cornish et al. (1979a) rejected previous contradictory results presented (loc. cit.), and ascribed irregularities in Spoor's (1976) data to misclassification. However, this seems unreasonable as there are so many irregularities and such a high proportion of incompatible crosses that subdivision of compatible reactions could not alter the number of mating types (Østerbye et al., 1980).

Further evidence has been produced from two additional studies for more complex self-incompatibility systems in the Gramineae. An F_1 family of Lolium multiflorum (Hay, 1978) displayed twenty-six mating types and an incompatibility pattern very similar to that of

L. perenne presented by Spoor (1976). This is not surprising as the two species are closely related and are sometimes considered as subspecies (Naylor, 1960).

Although the data from Briza spicata (Murray, 1979) showed a much higher frequency of cross-compatibility than the Lolium results (Spoor, 1976; Hay, 1978) they also displayed plants which were reciprocally incompatible and yet when crossed to other plants behaved differently (in common with observations on L. perenne and L. multiflorum).

Recently, Østerbye et al. (1980) have suggested that these results which seem to indicate a three locus system can be interpreted if one locus is assumed to have undergone a duplication. If the resulting loci, S' and S'', maintain their original set of common alleles, and segregate independently a maximum of sixty-four genotypes would be produced in the F_1 if the parents had no alleles in common. The four alleles from the S' and S'' loci would act as if they still belonged to one locus and identity for one of these alleles, together with one from the Z locus would be sufficient to cause incompatibility between pollen and pistil. If the parents of the F_1 progeny had alleles in common, reciprocal differences in compatibility would result. The system, therefore, is similar to that postulated to operate in self-incompatible tetraploid grasses (Lundqvist, 1957; 1962b). Alternatively, such a mechanism (S'S''Z) could have originated by interspecific exchange of S-loci, which are homologous because of close relationships between the species, but during speciation have changed their chromosomal positions. Therefore, L. perenne, L. multiflorum and L. rigidum may have inter-mixed

their S-mechanisms in this way during their evolution (Østerbye et al., 1980).

2.1.6.4 Ranunculaceae and Chenopodiaceae

The existence of incompatibility systems controlled by three and four S-loci was first postulated by Lundqvist et al. (1973). Within F_1 families, diallel sibcross matings resulted in high numbers of different mating types (in some cases more than the sixteen expected when self-incompatibility is controlled by two S-loci with gametophytic determination of pollen behaviour, and no alleles common to parents of the F_1 family). There was a high frequency of cross-compatibility which indicated that the loci were co-operating (as in the widespread two locus system found in the Gramineae). In addition, there were three levels of one way compatibility which could be explained by two S-loci (Fig. 2.3) but the lowest level would correspond to both loci being homozygous. Plants of this type would therefore be unable to fertilise either of the parent plants. However, as this expectation was not realised the three levels of one way compatibility in Ranunculus acris and the four levels of one way compatibility in Beta vulgaris were proposed to correspond to the number of heterozygous S-loci present in a plant. Therefore, three levels of one way compatibility would correspond to heterozygosity at one, two or three S-loci and plants from all levels could successfully pollinate both parents. For an incompatible reaction to occur it was postulated that all the alleles present in the pollen would have to be matched in the pistil. (Although gametophytic pollen behaviour was indicated, it was not initially proved to be present in either species).

The observation of the involvement of large numbers of incompatibility loci in complementary interaction supports the theory that this interaction depends on simple additive relationships between loci (Lundqvist, 1964b).

It is possible that, due to inbreeding, a population becomes homozygous for a single allele at one locus and the presence of this "hidden locus" would not be revealed by a sib-crossing scheme. This may be of importance in grasses, where two loci have already been detected and frequently investigations are based on samples from advanced generations that have been inbred. It is significant, in this context, that data from studies on the frequencies of self-fertility mutations in Secale cereale strongly indicated that an additional locus (as well as the two implicated in the genetical control of self-incompatibility) was involved in the formation of the incompatibility barrier (Lundqvist, 1968).

The breeding efficiency of a species where the genetical control of self-incompatibility is the result of the complementary interaction of several S-loci will be superior to that of a species where two loci control the self-incompatibility system. The number of alleles will be more efficiently used by the population when distributed over several loci but polyploidy would reduce the amount of cross-compatibility within a population (assuming the grass system where, in tetraploids, only one allele from each locus needs to be matched to produce an incompatible reaction).

Further research with R. acris confirmed that pollen behaviour was gametophytically determined (Østerbye, 1975) and that an additional fourth locus was involved in the self-incompatibility system within

a German population of this species (Østerbye, 1977). As the original Swedish plants were collected in the same locality and were shown to have alleles in common, crossing to the German plants may, in the future, reveal the presence of a fourth non-segregating locus represented by only one allele in the Swedish material (Østerbye, 1977).

The hypotheses advanced by Lundqvist et al. (1973) for the genetical control of self-incompatibility in Beta vulgaris by four S-loci were confirmed and extended by Larsen (1974; 1977a). The four S-loci showed complementary interaction and all the alleles in the pollen had to be matched in the pistil to produce an incompatible reaction. Evidence was later presented which indicated that all four loci belonged to one linkage group (Larsen, 1978a). This could have been brought about by either:

(i) a duplication of the original locus; or

(ii) co-operation between the originally unlinked loci leading to their evolution within one group.

Additional observations indicated a low number of alleles in the four loci, possibly only eight alleles in total (Larsen, 1978b). An inverse relationship was therefore proposed between the number of loci and the number of S-alleles in self-incompatibility systems (Larsen, 1978b).

Following the results and conclusions reached during studies on the genetical control of self-incompatibility in B. vulgaris, Lundqvist et al. (1973) and Larsen (1977a) reinterpreted Owen's (1942) data on self-incompatibility in B. vulgaris. Owen (1942) had originally proposed a two locus system, where the loci had

duplicated allelic series, gametophytic control of pollen specificities, and complementary interactions such that an incompatible reaction occurred when the alleles carried in the pollen grain were matched in the pistil by alleles from either or both allelic series. However, the anomalies in the data can be explained by the assumption of at least three gametophytic S-loci (Lundqvist et al., 1973; Larsen, 1977a). Larsen (1977a) also presented a re-interpretation of the genetic control of self-incompatibility in various other species. In each case Larsen (1977a) claimed that a more satisfactory explanation of the results was achieved by using a multilocus hypothesis.

Multilocus control of self-incompatibility has been found in relatively few species, a fact that Larsen (1977a) considered to be due to the choice of working hypothesis, materials and methods and because "many authors are captivated by the simplicity of the one locus model so that it becomes a dogma".

2.1.7 Evolution of self-incompatibility systems

The rapid evolution of the angiosperms during the cretaceous period was probably caused by the development of devices which prevented self-fertilisation and promoted more efficient cross-pollination than was possible in gymnosperms (Whitehouse, 1950). Outbreeding mechanisms such as dioecism and dichogamy are present in both phyla and it seems probable that the occurrence and evolution of self-incompatibility may have coincided with the rapid increase of flowering plants. Grant (1949) suggested that the development of an efficient outbreeding mechanism was likely to have occurred in conjunction with the evolution of specialised pollinating insects

and therefore must have arisen at a very early date in the history of the angiosperms. In addition, self-incompatibility systems are widespread throughout the plant kingdom occurring in more than 3,000 species from 250 genera belonging to approximately 70 families (Brewbaker, 1959; Linskens and Kroh, 1967). Therefore, it is reasonable to assume that incompatibility may have been present in the early ancestors of flowering plants (de Nettancourt, 1977).

Whitehouse (1950) proposed that self-incompatibility arose only once during the early history of the angiosperms. It has been suggested that gametophytic single locus polyallelic incompatibility systems were the first outbreeding mechanisms, and that sporophytic systems, heteromophism and self-fertility are all derived conditions (Whitehouse, 1950; Brewbaker, 1957; 1959; Pandey, 1960). In contrast, Bateman (1952) considered that self-incompatibility had evolved progressively and had arisen de novo more than once. The first type of incompatibility, according to Bateman (1952), was a weak polygenic system, and the efficiency of this system could have been improved by:

(i) the selection of non-specific modifiers increasing the efficiency of all loci; or

(ii) specific modifiers which would increase the effectiveness of one or two loci at the expense of the rest.

The two locus gametophytic systems of incompatibility found in the Solanaceae and Gramineae were generally thought to have evolved from a single locus gametophytic system as a result of a duplication of the S-locus (Lundqvist, 1962b; Pandey, 1962a). However, Hayman (1956) suggested that both loci were originally present or that one

locus became involved later without derivation from the original locus. In this way the loci could act in a complementary fashion and this function would not have to evolve.

The discovery of complex, multilocus control of self-incompatibility in the dicot families Ranunculaceae and Chenopodiaceae (Lundqvist et al., 1973; Larsen, 1974; 1977a; Østerbye, 1975; 1977) has challenged the arguments for a gametophytic polyallelic single locus system as the ancestor of all self-incompatibility systems. Self-incompatibility was investigated in species belonging to the Chenopodiaceae and Ranunculaceae as these families were thought to share a common ancestor with the monocot family, the Gramineae, in which complex systems of incompatibility have been demonstrated (Takhtajan, 1969). It is possible, therefore, that a complex type of incompatibility system was already present in angiosperms before the divergence of the investigated monocots and dicots, since a more complex type of control has been found in the primitive dicots than in the more advanced monocot species (Lundqvist et al., 1973). This discovery also supports the hypothesis that self-incompatibility systems are ancient constituents of the breeding system of angiosperms (Lundqvist, 1975).

As a result, the duplicative origin of the two locus "grass system" is in doubt and it has been proposed that the one locus system is the derived condition with loci: either becoming so tightly linked that they cannot be detected as distinct; or becoming homozygous for one allele in a population which remains hidden during sib mating studies (Lundqvist, 1975; Østerbye, 1975; Larsen, 1977a). In small populations, two and three locus gametophytic systems are less stable than a one locus system and the three locus system appears

to be particularly susceptible to fixation at one locus if all alleles are not equivalent in their contribution to fitness (de Nettancourt, 1977). Hence, the single locus system may have evolved from a multilocus system and is not necessarily the ancestral self-incompatibility system. Therefore, the discovery of multilocus systems of incompatibility provided evidence in support of Batemans (1952) hypothesis in which a weak polygenic incompatibility system was postulated to have arisen more than once during the course of evolution.

2.2 Physiology and biochemistry of the pollen-pistil interaction in self-incompatible species

In flowering plants the haploid male gametophyte interacts with the diploid sporophytic tissue of the pistil before liberating the male gametes in the vicinity of the female gamete. During this gametophyte-sporophyte interaction the male gametes are effectively screened by the pistil, thus ensuring the entry of only the appropriate pollen tubes into the embryo sac.

In the majority of species, the receptive surface of the pistil (the stigma), can be classified into two types depending on the presence or absence of exudate at the time of pollination. Species having sporophytic self-incompatibility systems were usually found to possess dry stigmas and species with gametophytic self-incompatibility systems generally had wet stigmas (Heslop-Harrison, 1975). There are two noteable exceptions to this generalisation: the Gramineae and Oenothera species combine gametophytic self-incompatibility and dry stigmas. It has been demonstrated that both types of stigma possess surface proteins (Heslop-Harrison and Shivanna, 1977; Shivanna, 1979). In the wet type of stigma proteins were present as a component of the exudate, and in the dry type proteins were present in the form of a hydrated layer, the pellicle (Heslop-Harrison and Shivanna, 1977).

In species with sporophytic self-incompatibility systems proteins from the pollen which are involved in the incompatibility response have been shown to be derived from the exine (Heslop-Harrison et al., 1973; Heslop-Harrison et al., 1974). During pollen maturation material derived from the tapetum (a sporophytic tissue) was observed to be deposited in cavities in the exine (Dickinson and Lewis, 1973; Heslop-Harrison et al., 1974).

In the majority of taxa having gametophytic self-incompatibility systems, incompatible pollen tubes are inhibited in the style. Since there is no interaction between incompatible and compatible pollen tubes growing down the same style it is probable that the incompatibility reaction takes place on the surface of the pollen tube and that the proteins involved are either held in the male gametophyte or synthesised during tube growth (Heslop-Harrison, 1978).

However, in those species having gametophytic self-incompatibility systems and stigmatic inhibition of incompatible pollen tubes, (Gramineae, Oenothera spp.) it is thought that S-allele products (proteins) are present in the wall of the pollen grain, the most probable site being the intine which was observed to receive gametophytic products during the early period of wall thickening (Heslop-Harrison et al., 1973; Heslop-Harrison, 1978; Knox and Heslop-Harrison, 1971).

2.2.1 Pollen-pistil interaction

It has generally been assumed that the primary event in the angiosperm self-incompatibility response must be a "recognition" reaction in which S-allele specific proteins are likely to be involved, and that this must be followed by secondary events which determine how the interacting partners, pollen and pistil, behave. It has been demonstrated for several species that pollen proteins bind to stigma proteins following pollination (Knox et al., 1976), but there has been no demonstration of the S-allele specificity of these proteins. After the primary recognition response, the secondary response of either acceptance (compatible reaction) or rejection (incompatible reaction) takes place.

Rejection may be the result of:

(i) an active inhibition of incompatible pollen tubes;

(ii) a passive stimulation of compatible tubes; or

(iii) a combination of these events (Heslop-Harrison, 1978).

2.2.2 Mechanism of acceptance/rejection

Despite the fact that many biochemical models of the self-incompatibility reaction have been proposed, little direct evidence has been obtained in support of any hypothesis. To be acceptable, a model must be consistent with the features known to characterise the phenomenon of self-incompatibility. One of the most important considerations is the tripartite structure of the S-locus, first proposed by Lewis (1960). The incompatibility gene is thought to consist of three linked segments; the "specificity" part which contains genetic information and is different for each S-allele, and two "activity" parts, one each for pollen and pistil.

The majority of models which have been proposed concern gametophytic single locus self-incompatibility systems, and may be classified into two distinct groups. The incompatibility reaction is either assumed to lead to a positive inhibition of pollen tube growth, or is equated with an immobilisation of growth substances (or the absence of suitable growth components).

The first hypothesis of inhibition of incompatible pollen tubes as a positive response was proposed by East in 1926 (cited by de Nettancourt, 1977), and was based on the antigen-antibody reaction in animals. The product of a specific S-allele in the pollen was postulated (East, 1926) to act as an antigen which is recognised during growth by an antibody produced by the same specific S-allele in the style, and pollen tube inhibition follows the recognition reaction. Linskens (1965) followed up this model,

although aware that antigen-antibody reactions are specific to animals, and proposed that the S-allele in the style codes, via an antibody synthesising unit, a Y antibody which can, when in excess, inhibit its own production (cross-pollination), or combine with an X protein in the pollen tube to form an inhibiting X-Y complex (self-pollination). Van der Donk (1975) developed the general antigen-antibody idea further and suggested that recognition depends on the matching of different substances produced by the pollen and stylar parts of the S-locus. Style specific polypeptides were postulated (van der Donk, 1975) to activate a set of genes necessary for pollen tube growth; pollen specific polypeptides inactivate style specific polypeptides when there is recognition between pollen and stylar polypeptides. Therefore, in an incompatible reaction the pollen specific polypeptides inactivate the style specific polypeptides which in turn are responsible for the cessation of pollen tube growth. In compatible reactions where there is no recognition, the style specific polypeptides promote pollen tube growth. The models of East (1926), Linskens (1965) and van der Donk (1975) are inconsistent with the fact that the specificity of the products of a given S-allele must be the same in the pollen and pistil.

Hypotheses in which the specificity of the products of an S-allele are identical in the pollen and pistil have been presented by Lewis (1965) and Ascher (1966). These models have been summarised by de Nettancourt (1972):

(i) a polypeptide is coded, in pollen and in style, by the specificity part of each S-allele present in the pollen and the style;

(ii) every different S-allele produces a different polypeptide;

(iii) after self-pollination, the polypeptides in the pollen tube dimerise, on the surface of the pollen tube, with identical polypeptides in the style to form a dimer repressor; and

(iv) the dimer repressor presumably switches off one or several loci responsible for pollen metabolism and pollen tube growth.

It should be noted that Lewis (1965) considered that only a tetramer would be physiologically active and suggested that identical polypeptides dimerise in the pollen and pistil and following self-pollination tetramerise, with the possible aid of glucose to form a tetramer regulator. This model has the advantage of facilitating a biochemical interpretation of the S and Z locus interaction in grasses. The general model presented above was further modified by Pandey (1975) to incorporate the tripartite structure of the S-locus postulated by Lewis (1960). Pandey (1975) suggested that the S-allele specific protein coded by the specificity part of the S-locus would be identical in pollen and pistil. This protein, it was postulated, would unite with the tissue specific complementary protein in the pollen and pistil. Therefore, this model explains the failure of stylar or pollen proteins to react amongst themselves but allows for the interaction between them to produce a repressor of the genes involved in pollen tube growth.

Despite the prevelance of "active" models a "passive" model has been proposed by Kroes (1973) who equated each S-allele in a polyallelic series to an absence of specific genetic information

in the pollen grains, and to the incapacity of the pollen to break down necessary growth complexes in the style. This model is not considered to provide a probable explanation of the mechanism of self-incompatibility, as the available evidence indicates an active participation by the S-alleles in pollen and pistil (Shivanna et al., 1978; Shivanna, 1979).

Direct evidence for the presence of S-allele specific proteins has been obtained from the use of immunological and electrophoretic techniques. In Brassica oleracea, S-allele specificity of stigma (but not of pollen) proteins has been demonstrated (Nasrallah and Wallace, 1967; Nasrallah et al., 1970; 1972). Nishio and Hinata (1977a, b), working with the same species, postulated that S-allele specificity in the stigma was expressed by a combination of protein fractions.

A biochemical model for the control of incompatibility where recognition reactions are determined by sporophytic fractions from the two parents and which takes place on the stigma has been outlined by Heslop-Harrison et al. (1975) and comprises:

(i) the synthesis of "recognition" proteins in the tapetum and their transfer to the exine during pollen maturation;

(ii) synthesis of "recognition" proteins in microbodies in the stigma papillae and their transfer to the stigma surface (pellicle);

(iii) interaction of exine and pellicle protein fractions following pollination;

(iv) activation of the male gametophyte and the stigma papilla leading to

(a) acceptance (tube emergence, cuticle penetration, tube growth, fertilisation)

or

(b) rejection (inhibitory responses in tube and papilla including callose synthesis).

It should be noted that one of the rejection responses common to both types of incompatibility systems is the deposition of callose. However, the place of deposition varies with the incompatibility system. In species with sporophytic self-incompatibility systems and stigmatic inhibition of incompatible pollen tubes, callose is deposited in pollen grains, pollen tubes and stigmatic papillae. In species with gametophytic self-incompatibility systems and stigmatic inhibition, callose is only deposited in the pollen grain or pollen tube. In species with gametophytic self-incompatibility but stylar inhibition, callose plugs frequently appear at the end of an incompatible pollen tube (de Nettancourt, 1977).

2.3 Interspecific incompatibility

In 1931 Anderson and de Winton noticed that crosses between self-incompatible and self-compatible Nicotiana species were only successful when the self-incompatible species was used as the pollinator. This was one of the earliest observations of the phenomenon of unilateral hybridisation which was later defined by Harrison and Derby (1955) as hybridisation "which can only be made in one direction, namely when the self-compatible species of the pair is used as the female parent and the self-incompatible one as the male parent". Unilateral incompatibility (as unilateral hybridisation was later termed) is the most common manifestation of interspecific incompatibility, which occurs in many plant groups and has been extensively reviewed by many authors (Lewis and Crowe, 1958; Abdalla and Hermsen, 1972; de Nettancourt, 1977).

The hypotheses which have been proposed to explain unilateral incompatibility have centered around three major points:

(i) interspecific and self-incompatibility are governed by different alleles or different elements of the S-locus (Lewis and Crowe, 1958; Pandey 1962b; 1968; 1969);

(ii) specific rejection genes interact with the S-locus to produce interspecific incompatibility (Abdalla and Hermsen, 1971); and

(iii) rejection genes involved in interspecific incompatibility are separate from and do not interact with the alleles of the S-locus (Hogenboom, 1973; 1975).

For this phenomenon of interspecific incompatibility Hogenboom (1973) introduced the term "incongruity" to distinguish it from self-incompatibility.

2.3.1 Interspecific hybrids in the genus Lolium

The most extensive study of hybridisation within the genus Lolium was conducted by Jenkin over several years (Jenkin, 1933; 1954; 1955b; Jenkin and Thomas, 1939). The results were presented as percentage seed set, percentage germination and fertility of the F_1 generation. The summarised results (adapted from Naylor, 1960) of crosses involving three outbreeding and three inbreeding species are presented in Table 2.3. Naylor (1960) concluded after studying Jenkin's results that intragroup crosses (SP x SP or XP x XP) produced a higher seed set than intergroup crosses. Table 2.3 does not show that there was a higher seed set when inbreeding species were crossed but seeds produced germinated more readily than those produced as a result of intergroup crosses. It is very difficult to cross inbreeding species as the anthers are poorly exserted and little pollen is shed; this may explain why a lower seed set was observed than might have been expected. Hybrid plants produced between inbreeding species were either male sterile, female sterile or had a very low level of fertility and could not be perpetuated (Table 2.3), thus indicating that the species are distinct. Hybrids produced as a result of the intergroup crosses (XP x SP or SP x XP) were all male sterile, and frequently plants died before reaching maturity. Fully fertile F_1 hybrids were obtained in crosses between outbreeding species and no breeding barrier operated in contrast to the interspecific barrier preventing

Table 2.3

Results of interspecific crosses within the genus Lolium (Adapted from Naylor, 1960)

♀	♂ SP (self-pollinated)			♂ XP (cross-pollinated)		
	% seed set	% germination	fertility F_1	% seed set	% germination	fertility F_1
SP	45.6	88.8	generally ♂ or ♀ sterile	54.2	4.2	♂ sterile ♀ fertile
XP	46.7	42.1	♂ sterile ♀ fertile	66.0	77.3	♂ fertile ♀ fertile

Key: SP = L. loliaceum, L. remotum, L. temulentum

XP = L. italicum, L. perenne, L. rigidum

hybridisation between inbreeding species (Table 2.3).

The male sterility observed in the intergroup hybrids was probably partly caused by the differences in chromosome size between inbreeding and outbreeding Lolium species (Naylor and Rees, 1958; Naylor, 1960; Rees and Jones, 1967). Inbreeding species were shown to have thirty to forty percent more DNA than outbreeders, in this genus, and as a result have larger chromosomes. The genetic significance of this extra DNA, which appears to be mainly repetitive base sequences is not known (Malik and Thomas, 1966). In a recent study Hutchinson et al. (1979) found that the extra DNA did not affect a wide variety of seedling and adult plant phenotype characteristics.

The fact that fertile F_1 hybrids are frequently obtained from crosses between the outbreeding Lolium species led Naylor (1960) (who studied the L. perenne x L. multiflorum hybrid and found no cytological unbalance) to propose that the three outbreeding species are in fact subspecies. In contrast, Jenkin (1954) regarded L. perenne and L. multiflorum as distinct but closely related species and Malik (1967) thought of L. multiflorum and L. rigidum in the same way. The evidence in support of separate species can be summarised as:

(i) all three species show different reactions when crossed to species in the genus Festuca (Jenkin, 1955a);

(ii) all three forms can be distinguished morphologically (Malik and Thomas, 1966); and

(iii) there is some evidence of chromosome differentiation (Malik and Thomas, 1966).

Although the genotypes considered in these studies (loc. cit.) may or may not have been representative of the species as a whole, it should be noted that Humphries (1980) in the Flora Europea has classified the three outbreeding species as separate and distinct, based on morphological characteristics.

Several studies on the evolution of the species within the genus Lolium have produced different conclusions. Essad (1962) (cited by Malik and Thomas, 1966), considered L. perenne to be the original species which, through progressive evolution, gave rise to forms like L. rigidum (annual, outbreeding) and hence to inbreeding species. Naylor (1960) considered the fact that inbreeders had larger chromosomes than outbreeders supported the theory (Darlington and Mather, 1949) that inbreeders evolved from outbreeders by a process of chromosome duplication and rearrangement. This theory was further supported by the recent study of Hutchison et al. (1979). Malik (1967) proposed that the ancestral species was outbreeding, annual and had medium sized chromosomes and that evolution was in two directions, producing inbreeding annuals with larger chromosomes and outbreeding species of various growth habits with smaller chromosomes. This hypothesis is supported by karyotype morphology as L. perenne and L. remotum have more advanced karyotypes (asymmetrical) than the remaining species (symmetrical karyotypes) (Stebbins, 1958).

2.3.1.1 Tetraploid hybrids

A wide range of tetraploid ryegrasses have been produced, such as the autotetraploids Barlatra (L. perenne) and Sabalan (L. multiflorum), and the allotetraploids Sabrina and Sabel (L. perenne and L. multiflorum). Diploid interspecific hybrids, although fertile, are genetically

highly unstable and potential interspecific allotetraploid hybrids are assessed genetically and cytologically as their commercial exploitation depends on their uniformity and stability over seed multiplication generations (Breese and Thomas, 1977).

Results presented by Clarke and Thomas (1976), Clarke (1978) and Lewis (1978) (Table 2.4) showed that allotetraploid hybrids had a lower level of multivalent chromosome pairing and a corresponding higher level of bivalent pairing than autotetraploids of either L. perenne or L. multiflorum. While this may indicate a certain degree of preferential pairing, bivalent pairing can be shown to be preferential only when it results in disomic segregation (Breese and Thomas, 1977). Although Breese and Thomas (1977) demonstrated partial disomic segregation in certain allotetraploid F_1 hybrids of L. multiflorum x L. perenne, Lewis (1978) concluded that in the allotetraploids he studied segregation was tetrasomic.

Table 2.4

Multivalents and bivalents per cell in auto- and allotetraploid hybrids of Lolium species

Species	Type of tetraploid	Multivalents per cell	Bivalents per cell	Reference
L. multiflorum	auto	3.93-4.39	9.96	Clarke and Thomas (1976)
L. perenne	auto	3.63	6.54	Lewis (1978)
Sabrina	allo	1.12-1.37	9.96-10.46	Clarke and Thomas (1976)
Double hybrid	allo	1.04-1.40	10.81-11.48	Clarke (1978)
F_1 hybrid	allo	2.42	8.98	Lewis (1978)

2.4 Practical applications of self-incompatibility

Self-incompatibility is common among cultivated plant species and, as such, can be considered a nuisance or an advantage to the plant breeder, depending on his aims and the production techniques employed. Before the incompatibility system can be utilised or eliminated the genetical control must be established for each species.

Possible breeding objectives may include:

(i) the introduction of incompatibility into cultivars or breeding lines from other lines or wild relatives;

(ii) permanent elimination of incompatibility by genetic means;

(iii) regulating the strength of the incompatibility reaction by genetic or physiological means, to ensure self-pollination in a certain line at a given time and cross-pollination at all other times; and

(iv) the development of breeding procedures which will allow the utilisation of incompatibility for hybrid seed production (Frankel and Galun, 1977).

The most important use of incompatibility has proved to be the production of F_1 hybrids. In theory, inbred S-homozygous plants (which are produced by selfing) are intercrossed. If the inbred lines display good combining ability the resulting F_1 hybrids will be superior to either parent. Single, double or triple crosses may be used depending on the self-incompatibility system involved and the part of the crop which is harvested (Reimann-Phillip, 1965; Duvick, 1966). Single cross hybrids are the most uniform but also

the most expensive to produce, while triple cross hybrids are the least uniform and the least expensive to produce. However, the latter cannot be utilised in species where pollen behaviour is determined gametophytically.

Of economically important crop species having a one locus gametophytic self-incompatibility system double cross F_1 hybrids have only been produced in Trifolium pratense (Anderson et al., 1972). The gametophytic self-incompatibility systems operating in the Gramineae have been found to be genetically controlled by two or three loci (loc. cit.). Heterosis has been observed in F_1 hybrids produced by controlled single crosses in Paspalum notatum and Cynodon dactylon (Burton and Hart, 1964). The processes involved in producing these F_1 hybrids were laborious and expensive. The structure of flowers of forage grasses does not permit mass emasculation and male sterile lines, while commonly present, have not been actively sought and developed. Since these aids in the production of F_1 hybrids were unavailable England (1974) suggested a method for the production of F_1 hybrid seed in commercial quantities based on the two locus gametophytic self-incompatibility system. If more than two loci were found to control self-incompatibility in any species of forage grass the percentage of F_1 offspring expected to be hybrid would be reduced (2 loci : 83% hybrid; 3 loci : 71% ; 4 loci:64%) (England, 1974).

Within species displaying homomorphic sporophytic self-incompatibility systems, considerable success has been achieved in the production of F_1 hybrid cultivars (Frankel and Galun, 1977). One of the first such hybrids was a triple cross kale (Brassica

oleracea var. acephala) bred at Cambridge (Thompson, 1964).

Despite the simplicity of the theory there are problems associated with F_1 hybrid production in practice, including:

(i) new incompatibility specificities, which cause self-fertility are often produced as a result of inbreeding in species having gametophytic self-incompatibility;

(ii) depression, caused by continuous inbreeding, sometimes resulting in the loss of lines (this may be overcome by vegetative reproduction);

(iii) pseudo-compatibility, where weak S-alleles are incorporated into parental lines;

(iv) incompatibility may be reduced in certain environmental conditions (such as high temperature and high humidity); and

(v) the restriction of pollination within parental lines by vectors was observed when lines were morphologically distinct (Frankel and Galun, 1977).

In conclusion, it should be noted that in spite of these problems, sporophytic incompatibility has been successfully used for commercial hybrid seed production.

2.5 Pollen

Investigations of self-incompatibility systems require a plentiful supply of viable pollen over a period of time. Unfortunately, Gramineae pollen is not amenable to storage using conventional methods (section 2.5.2), and pollen that is collected must be used within hours. Therefore, a reliable method for assessing pollen viability rapidly and accurately is essential.

2.5.1 Pollen viability

Over the years many methods have been developed to demonstrate pollen viability including specific stains and the ability of pollen to germinate in culture media (Manthiratna and Hayward, 1973). Although the best estimate of viability is the ability of pollen to achieve fertilisation *in vivo* this method is often unsatisfactory because of the time lag between pollination and seed set, and pollen which is incompatible can be scored as inviable.

2.5.1.1 Stains

In staining pollen grains to assess viability, chemicals are adsorbed by specific cell constituents present in mature pollen. Frequently, immature or aborted pollen grains contain levels of these chemicals sufficient to yield positive results.

2.5.1.1.1 Cotton blue in lactophenol demonstrates

potential viability by staining the callose lining of intact pollen grains (Manthiratna and Hayward, 1973).

2.5.1.1.2 Nitroblue tetrazolium indicates the

degree of succinic acid dehydrogenase activity in pollen cytoplasm. The stain, nitroblue tetrazolium, is an indicator dye and reacts with succinic acid, if present, to produce a coloured compound, formazan. Succinic acid dehydrogenase is an enzyme involved in the

Krebs Cycle, and its presence has been equated with the capacity for oxidative metabolism and hence viability (Hauser and Morrison, 1964). It has been shown, using pollen from a common source, that pollen stained with nitroblue tetrazolium salts gave a lower percentage of viable grains than pollen stained with cotton blue in lactophenol. This indicated that although the lining of the pollen grain was intact they did not all have the ability to respire (Hauser and Morrison, 1964; Manthiratna and Hayward, 1973).

2.5.1.1.3 Fluorescein diacetate estimates pollen viability by enzymatically induced fluorescence. The fluorochromatic reaction depends on the entry of non-polar substrate into the vegetative cell where it is hydrolysed by esterase to give the polar product, fluorescein, which is retained by the cell membrane. The property primarily tested for, apart from the presence of an active esterase, is the integrity of the plasmalemma. Normal permeability of the plasmalemma was considered to be closely correlated with pollen viability (Heslop-Harrison and Heslop-Harrison, 1970). Heslop-Harrison and Heslop-Harrison (1970) demonstrated that following treatments affecting the cell membrane (such as the addition of saponin, heating or puncturing the pollen grain) the fluorochromatic reaction did not develop in pollen grains immersed in sclutions of fluorescein diacetate.

2.5.1.2 Germination <u>in vitro</u>

The principle of an <u>in vitro</u> assay is to record the percentage germination after a given period of time. This is the standard test for pollen viability and is based on the assumption that

germination *in vitro* approximates that *in vivo*.

Angiosperm pollen grains are either binucleate or trinucleate at anthesis (Brewbaker, 1957): grass pollen is trinucleate (Brewbaker, 1957; 1959). Results generally showed that trinucleate pollen is difficult to culture on artificial media (Brewbaker and Majumder, 1959). Despite this, successful germination of Gramineae pollen *in vitro* has been reported for *Pennisetum thyphoideum* (Vasil, 1960), *Secale cereale* (Pfahler, 1965; Shivanna, Heslop-Harrison and Heslop-Harrison, 1978) *Zea mays* (Cook and Walden, 1965; Pfahler, 1967; 1968) *Avena byzantina* C. Koch (Wallace and Karbassi, 1968) *Lolium perenne* and *L. multiflorum* (Ahloowalia, 1973; Manthiratna and Hayward, 1973). All the culture media used for *in vitro* germination tests contained high concentrations of sugar (usually sucrose). In general, trinucleate pollen grains required a higher concentration of sucrose to germinate *in vitro* than binucleate pollen grains (Brewbaker, 1959). In all the successful attempts to germinate grass pollen *in vitro*, boron, present as boric acid, was included in the media. Boron has been known to stimulate germination and pollen tube growth *in vitro* since the early 1930's (Schmucker, 1935). However, the function of boron is not known, although many roles have been postulated (Visser, 1955; Pfahler, 1968). Calcium is also considered necessary for pollen germination and pollen tube growth *in vitro* by some authors (Pfahler, 1967; Brewbaker and Kwack, 1963). Calcium, diffusing out of pollen grains was thought to stimulate others to germinate, producing the so-called "population effect" (Brewbaker and Kwack, 1963). Both calcium and boron were toxic to pollen grains at high concentrations and caused a decrease

in germination percentage and a decrease in pollen tube length *in vitro* (Vasil, 1960; Pfahler, 1967; 1968). Physical conditions as well as the chemical constituents of the medium were observed to affect germination *in vitro*. Pfahler (1965) and Wallace and Karbassi (1968) noted that a high relative humidity above the culture medium increased the percentage germination of pollen grains *in vitro*.

Following germination, many workers have measured the lengths of pollen tubes. Usually, the length to which a pollen tube grew *in vitro* was found to be insufficient to achieve fertilisation *in vivo* (Brewbaker, 1959; Rosen, 1971; Pfahler, 1965). It is unlikely that the solid or semi-solid media used for germination tests *in vitro* resemble the pistil, especially in the Gramineae and it must be concluded that optimum growth conditions are rarely, if ever, established in *in vitro* media. The pistil provides the perfect environment during compatible matings which has not, or cannot be duplicated (Rosen, 1971; Stanley and Linskens, 1974).

2.5.2 Pollen storage

Reliable methods for storing pollen would be of use to plant breeders by extending the crossing season and allowing easier interspecific hybridisation between species flowering at different times.

The maintenance of pollen viability depends on the conditions of storage. The most important factors include relative humidity, temperature, and the constituents of the atmosphere surrounding the pollen. As well as being difficult to germinate *in vitro*, grass pollen is short lived and most workers have found that it is not amenable to storage in conditions of low temperature and low relative

humidity (Visser, 1955; Brewbaker and Majumder, 1959).

Gramineae pollen was shown to lose its viability within one day under dry storage conditions (less than 30% relative humidity) (Linskens, 1964; Stanley and Linskens, 1974). This loss was slightly slower at low temperatures (Antony and Harlan, 1920). For example, Zea mays pollen stored at high relative humidity (95%) and low temperature (2°C) remained viable for four to five days (Pfahler and Linskens, 1973) or twelve days (Walden, 1967). Grass pollen could not be stored for longer periods of time by freeze drying or vacuum drying (Antony and Harlan, 1920; Stanley and Linskens, 1974).

The short lived nature of Gramineae pollen has been attributed to its less resistant morphological structure as the exine provides no protection against dessication during storage (Linskens, 1964). In addition, it is probable that trinucleate pollen grains contain few metabolites as a result of the second nuclear division occurring before dehiscence, a condition which may contribute to their reduced longevity compared to binucleate pollen grains (Brewbaker, 1959).

3. MATERIAL AND METHODS

3. MATERIAL AND METHODS

3.1 Material

The material used in this study is detailed below.

Species	Origin
L. rigidum, Gaud. (2x) Seed from three populations collected in France	Station National d'essais de semences, Versailles
L. multiflorum, L. (2x) S22, Sceemster, Combita	Commercial varieties
L. multiflorum, L. (4x) Experimental lines Bb 1353, Bb 1419	Welsh Plant Breeding Station, Aberystwyth
L. perenne, L. (2x) Experimental lines Ba 8979, Ba 8981	Welsh Plant Breeding Station, Aberystwyth
L. perenne, L. (4x) Experimental lines Ba 8982, Ba 8983	Welsh Plant Breeding Station, Aberystwyth

3.1.1 Composition of F_1 families

The parentage of the F_1 families used in this study are detailed in Table 3.1.

Table 3.1

Parentage of F_1 families

Species	Parents	F_1 family code
L. rigidum (2x)	Plants from 3 populations (coded B,0,L) crossed	B5x08 01x02 L3xB1
L. multiflorum (2x)	S22 x Sceemster Sceemster x Combita S22 x Combita	ED1 ED2 ED3
L. multiflorum (4x)	Bb 1419 x Bb 1419	Lm3, Lm4
L. perenne (2x)	Ba 8979 x Ba 8981	M1, M2
L. perenne (4x)	Ba 8982 x Ba 8983 Ba 8982 x Ba 8982	Lp1 Lp2
L. perenne x *L. multiflorum* interspecific hybrid (4x)	Ba 8982 x Bb 1419	PM1, PM2

NB In all crosses involving plants from experimental lines, different plants from each line were used for each cross.

3.2 Methods

3.2.1 Production of F_1 families

F_1 families were produced by controlled pollinations between self-incompatible, unrelated plants. Seeds were germinated on moist filter paper in petri dishes and sown in Fisons potting compost in 4" pots when 7-10 days old (in this way only one plant per pot became established). The progeny, of 30-40 plants per family, were grown in a greenhouse until flowering. Vernalisation, where necessary, was accomplished by leaving the plants in an outdoor cage for 12 weeks. Flowering could be achieved out of season by the use of a 16 hour daylength in a heated greenhouse. Some families were allowed to flower naturally. The timetable of events for each family from production to use is given in Table 3.2.

3.2.2 Diallel cross

Pollinations were performed *in vitro* using Lundqvists (1961b) petri dish technique. Whole pistils were dissected from florets immediately prior to anthesis, and the base of the ovary was placed in a recorded position on an agar plate. In this way two pistils per plant of up to forty plants were able to be pollinated by one male plant. Each petri dish was therefore equivalent to a single male array of a diallel cross, and each array was replicated. Stigmas were pollinated within eight hours of plating.

Fresh pollen was collected by bagging cut flowering heads, just beginning to shed pollen, which were placed under lights to encourage anther exsertion. After a maximum of two hours the bags were tapped to shatter anthers which had not burst and pollen was dusted over the pistils. The plates were incubated for six hours

Table 3.2

Timing of events for each F_1 family from production to flowering

Species	Ploidy	Code	Date production	Date sowing	Vernalisation	Flowering	Date diallel
L. rigidum	2x	B5x08	May 1978	Sept 78	-	forced	Jan-Mar 79
		01x02	" "	" "	-	"	Feb-Mar 79
		L3xB1	" "	Aug 79	-	"	Jan-Mar 80
L. multiflorum	2x	ED1	Jan-March 1977	May 77	-	natural	July-Aug 78
		ED2	"	" "	-	forced	Apr-Jun 78
		ED3	"	" "	-	"	Jan-Mar 78
	4x	Lm3	Jun 1978	Oct 78	-	forced	Mar-May 79
		Lm4	" "	" "	-	natural	Jun-Aug 79
L. perenne	2x	M1	Jun 1978	Jan 79	Winter 79/80	forced	May-Jun 80
		M2	" "	" "	Spring 1980	died due to frosting when too leafy	
	4x	Lp1	Jun 1978	Oct 78	Winter 78/79	natural	July-Aug 79
		Lp2	" "	" "	Winter 79/80	forced	Apr-May 80
L. perenne x L. multiflorum interspecific hybrids	4x	PM1	Jun 1978	Jan 79	-	natural	Aug-Oct 79
		PM2	" "	" "	Autumn 1979	natural) & forced)	Sept-Oct 79 * Mar-Apr 80 *

Key: * used twice

at room temperature, this being sufficient time to allow germination and pollen tube growth to the base of the stigma, in compatible crosses. The petri dishes were then stored under refrigeration (0-4°C) for a maximum of 48 hours before staining.

3.2.2.1 Agar for plating pistils

A nutrient agar comprising 100g sucrose, 30g lima-bean agar (Difco), and 0.1g boric acid per litre of distilled water was prepared and autoclaved at 15lbs p.s.i. for 15 minutes at 121°C. After cooling, the agar was poured into petri dishes under sterile conditions.

3.2.2.2 Staining pistils to observe pollen tube growth

Several staining techniques were examined (Lalouette, 1967; Kho and Baer, 1968; Sherwood and Vance, 1976; Eschrich and Currier, 1974) in order to establish the most suitable for the material studied. The basis of each technique was to use aniline blue to stain for callose, which plugs and lines pollen tubes, and to observe the resulting fluorescence using ultra violet (U.V.) illumination.

3.2.2.2.1 Staining schedule. The most satisfactory staining technique was that devised by Martin (1959), and modified by Lawrence (p.c., 1978). Whole pistils were stained on a slide in a 0.2% solution of water-soluble aniline blue (Gurr) in 0.1M K_3PO_4 (tri-potassium orthophosphate) for 1-2 minutes. Excess stain was removed and the pistils were mounted in glycerol and crushed gently under a coverslip. Observations were made using a Leitz Ortholux 2 fluorescent microscope with incident light illumination.

3.2.2.2.2 Fluorescence microscopy. The difference between fluorescence and light microscopy is in the light source.

For fluorescence microscopy a high pressure mercury vapour lamp is used, which supplies radiation in the U.V. and visible short wave part of the spectrum. Exciting filters are used in conjunction with the light source to provide only the wavelengths required for fluorescence excitation (blue light 390-400 um; U.V. 360 um) and transmit them to the object. Fluorescence intensity is slightly greater in blue light but the fluorescence obtained in not as specific as that observed in U.V. light (Eschrich and Currier, 1964). To avoid reduction of contrast, the primary fluorescence of the optical elements of the microscope, slide, coverslip and mounting fluid are kept as low as possible. By use of a second set of filters (suppression filters), short wavelengths scattered by the specimen are absorbed and only the long wavelengths of the fluorescent parts of the tissue are transmitted.

The Ortholux 2 was fitted with a Wotan HBO 100W super pressure mercury lamp and two filter systems.

(i) Filter system A for U.V. excitation which resulted in a yellow fluorescence:

exciting filter	2x2mm UG1
dichroic mirror	Tk 400
suppression filter	K 430

(ii) Filter system G for blue illumination which resulted in a green-yellow fluorescence:

exciting filter	3mm BG12
dichroic mirror	Tk 510
suppression filter	K 515

3.2.3 Pollen viability

Pollen viability was determined by two alternative methods: the ability of pollen to take up a specific stain; and the ability of pollen to germinate *in vitro*.

3.2.3.1 Stain for pollen viability (Heslop-Harrison and Heslop-Harrison, 1970)

A stock solution of fluorescein diacetate (2mg fluorescein diacetate (Sigma)/ml acetone) was prepared and stored under refrigeration (0-4°C). Aqueous solutions of fluorescein diacetate flocculate at concentrations above 5×10^{-6}M and therefore fresh substrate solutions were prepared each time pollen viability determinations were made. Substrate solutions were prepared by adding stock solution drop by drop to approximately 20ml 0.5M sucrose until the solution appeared milky. A range of sucrose solutions were tested to find the tonicity which gave the fewest number of burst pollen grains.

For each plant tested three anthers immediately prior to anthesis were dissected from a floret and gently squeezed over a drop of solution on a slide. A coverslip was placed on the drop and the preparation was viewed under U.V. illumination on a Leitz Ortholux 2 fluorescent microscope. Pollen grains which fluoresced were scored as viable. Anthers from two different florets from separate spikelets for each plant tested were used, and each test was replicated. Between 150 and 200 pollen grains were scored for each preparation.

3.2.3.2 Pollen grain germination *in vitro*

Anthers immediately prior to anthesis were gently teased open over a sterile semi-solid medium in petri dishes comprising 1% Davis

Standard agar, 0.6M sucrose, 10^{-3}M boric acid and 2.5×10^{-3}M calcium chloride (Shivanna, Heslop-Harrison and Heslop-Harrison, 1978). Petri dishes and pollen grains were incubated for one hour at room temperature. Pollen grain diameter and the corresponding pollen tube length, for at least 15 grains, were measured using a micrometer eyepiece fitted to the x10 objective of a Vickers M15c light microscope.

3.2.4 Anther and stigma length

The length of anthers immediately prior to anthesis was measured using vernier calipers. At least 50 anthers per plant were measured, from representative plants within a family. The size of stigmas corresponding to the anthers measured was also determined using vernier calipers. Whole pistils were plated onto agar (20% Davis Standard agar) and left for 30 minutes at room temperature for the feathery stigmas to achieve maximum extension. At least 20 stigmas (both branches) were measured per plant.

3.2.5 Cytology

The chromosome number of plants in the F_1 families was determined using the following technique.

3.2.5.1 Pollen mother cell meiosis

Young influorescences were fixed in 3:1 absolute alcohol : acetic acid for 24 hours at 4°C, then transferred to 70% alcohol and stored under refrigeration until required. Influorescences were hydrolysed in N hydrochloric acid (HCl) for 6 minutes at 60°C, and then stained in Feulgen at 4°C for one hour. Anthers were dissected from florets and stained in acetic orcein for 1-2 minutes, on a slide, then macerated and squashed under a coverslip. Preparations were viewed using a Reichart Biopan microscope.

Permanent preparations were made by freezing with Polar Spray (dichlorodifluoromethane), then the coverslip was removed and the preparation was dehydrated in absolute alcohol for 90 seconds, air-dried and mounted in Euparal under a clean coverslip.

3.2.5.1.1 Feulgen (Darlington and LaCour, 1942). One gram basic fuchsin (B.D.H.) was dissolved in 200ml boiling distilled water. The solution was shaken and cooled to 50°C, then filtered. Thirty ml HCl and 3g potassium disulphide ($K_2S_2O_4$) were added to the filtrate and the solution was allowed to bleach for 24 hours in a tightly stoppered brown bottle in the dark. Then 0.5g decolourising carbon was added to the bleached solution which was shaken well for one minute. The solution was filtered through coarse filter paper and the filtrate was stored in a brown bottle at 4°C.

3.2.5.1.2 Acetic orcein (Darlington and LaCour, 1942). One gram of orcein (Raymond A. Lamb) was dissolved in 45ml hot glacial acetic acid. When cool, 55ml distilled water was added. The solution was shaken, then filtered and stored in a brown dropper bottle.

4. RESULTS

4. RESULTS

4.1 F_1 families

The results of the diallel crosses carried out in thirteen F_1 families of ryegrass are given in detail in Figures 4.1 to 4.13. A detailed summary of the species, ploidy, code and number of plants in each F_1 family is given in Table 4.1.

In all the F_1 families pollen control of the incompatibility reaction was found to be determined gametophytically as differential pollen behaviour was observed on stigmas in compatible crosses. In species where the incompatibility system is determined sporophytically differential pollen behaviour does not occur; pollen is either all compatible or all incompatible. Although the pollen behaviour was determined gametophytically it was not possible to classify pollen into classes such as 50%, 75% or 100% compatible. However, completely incompatible crosses (0% compatible) were easy to score.

Each F_1 family contained plants which were self-compatible when excised stigmas were pollinated *in vitro*. The proportion of self-compatible plants ranged from 7.4% in a diploid *L. multiflorum* family (ED2: Fig. 4.5) to 52.2% in a diploid *L. perenne* family (M1: Fig. 4.9). In each pollination classified as self-compatible *in vitro* only one or two pollen tubes were observed growing through the stigma and the majority of pollen grains were incompatible. In contrast, numerous pollen tubes grew through stigmas in cross-compatible reactions. When flowering heads were selfed by being bagged singly very few seeds were set (Table 4.2). There did not appear to be any correlation between seed set by bagging flowering heads and pollen tube growth through self stigmas *in vitro*. It is

Table 4.1

Details of F_1 families

Species	Ploidy	Code	No. plants in family	Figure
L. rigidum	2x	B5x08	25	4.1
		01x02	25	4.2
		L3xB1	27	4.3
L. multiflorum	2x	ED1	26	4.4
		ED2	27	4.5
		ED3	17	4.6
	4x	Lm3	24	4.7
		Lm4	28	4.8
L. perenne	2x	M1	23	4.9
	4x	Lp1	25	4.10
		Lp2	38	4.11(a)(b)
L. perenne x *L. multiflorum* interspecific hybrid	4x	PM1	20	4.12
		PM2	13	4.13

Table 4.2

Results of selfing plants in vivo (no. seeds) and in vitro (± pollen tube growth) for nine F_1 families

Family:	B5x08		01x02		L3xB1		ED1		ED3	
	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±
Plant										
1	0	-			0	-				-
2	0	-			0	-				
3	0	-			0	-	0	-		-
4				-						
5	0	+			0	-		-		
6	0	-		-	0	-			0	+
7	0	+			0	-			12	-
8		-	0	-	0	-				
9					0	-	0	-	0	-
10		-		-	0	-				
11		-			0	-				
12		-			0	-				
13	0	-		-	0	-		-	0	-
14	0	-		-	0	-	1	-		-
15				-				-	10	-
16	1	-			0	+				
17	0	-			0	-				
18	0	-			0	+		-	0	-
19	0	-	0	+	0	-		+		
20					0	-	5	+		-
21	0	-	0	+	0	-	1	-		
22		+	0	+	0	-		+	0	-

Table 4.2 contd.

Results of selfing plants in vivo (no. seeds) and in vitro (± pollen tube growth) for nine F_1 families

Family:	B5x08		01x02		L3xB1		ED1		ED3	
	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±
Plant										
23				-	0	-				
24			0	+	0	-	0	+	7	+
25			0	-	0	-				
26	0	-		-	0	-	1	-		
27	0	-	0	-	0	+	2	+	0	-
28	0	-	0	-	0	-		+	3	-
29			0	+	0	-		-	0	+
30	0	-		-			0	+		
31	0	+	0	-			0	-	0	-
32				+				-		
33			0	-			2	+		
34	0	+	0	-			4	-		-
35			0	-				-		
36				-				-		
37				-						
38		-						-		
39								-		
40								-		

Key: + self-compatible - self-incompatible

B5x08, 01x02, L3xB1 } *L. rigidum*, diploid

ED1, ED3 } *L. multiflorum*, diploid

Table 4.2 contd.

Results of selfing plants in vivo (no. seeds) and in vitro (± pollen tube growth) for nine F_1 families

Family:	Lm3		Lm4		M1		Lp2	
	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±
Plant								
1			0	-			0	-
2			2	+				-
3	5	-	0	-			0	-
4					8	-		-
5		-	0	-	2	+	0	-
6	0	-	0	-			0	-
7	0	-	1	-	0	-	0	-
8	0	+	1	-				-
9	0	-					3	-
10			1	-	0	-	0	-
11	2	-			0	-	0	+
12			1	-	2	-		+
13					1	-		-
14		-	0	-			0	-
15			1	-			0	+
16		+		+	0	-		+
17					0	+	0	-
18	0	+	0	-			8	-
19	0	-			0	-	0	+
20			1	-	0	+	0	+
21							0	-
22	0	+	0	-			0	+

Table 4.2 contd.

Results of selfing plants in vivo (no. seeds) and in vitro (± pollen tube growth) for nine F_1 families

Family:	Lm3		Lm4		M1		Lp2	
	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±	in vivo no. seeds	in vitro ±
Plant								
23	0	-	0	-	0	-	0	+
24			0	+	0	+		
25		-	0	-	0	+	0	-
26			0	+	0	+	0	+
27	0	+						-
28					0	-		-
29	0	-	1	-	0	-	0	-
30	0	-	2	+	0	+		-
31	0	-	3	-				-
32	0	+	0	-	0	+	1	+
33			2	-	0	+	0	-
34					0	+	0	-
35	1	+	0	+	0	+	0	-
36	0	-	2	+	0	+	0	-
37		-					0	-
38	0	-	2	-				
39							0	+
40	0	-	0	-			0	-

Key: + self-compatible - self-incompatible

Lm3) Lm4) L. multiflorum, tetraploid

M1 L. perenne, diploid

Lp2 L. perenne, tetraploid

possible that, on occasion, the environment in the petri dish (high temperature and relative humidity) was conducive to selfing.

The frequency of pollinations which were cross-incompatible was 22% for diploid F_1 families and 26% for tetraploid F_1 families (Table 4.3; Appendix 1, Table 1). Cross-incompatible reactions between plants were of two types. Firstly, examples of one way compatibility were found in each diallel, such that the cross plant A x plant B was compatible and plant B x plant A was incompatible. The phenomenon of reciprocal differences between plants is characteristic of sporophytic self-incompatibility systems (and is caused by dominance of S-alleles), and multilocus gametophytic self-incompatibility systems when the parents of the F_1 family have alleles in common. The proportion of pairs of crosses recorded as one way compatible ranged from 7.3% in L3xB1 (diploid, annual: Fig. 4.3) to 43.8% in Lm3 (tetraploid, biennial: Fig. 4.7) (Table 4.4). Secondly, there were plants which were reciprocally incompatible but had differing reactions with other plants, thus indicating that they did not belong to the same mating group. There was only one example in all of the thirteen F_1 families where two reciprocally incompatible plants had the same reactions with all other plants in the diallel: plants 16 and 27 in family PM2 (tetraploid interspecific hybrid between L. perenne and L. multiflorum: Fig. 4.13). However, many of the possible crosses in this family have not been performed and it is likely that plants 16 and 27 had different mating types. Therefore, there were as many different mating types as plants in each F_1 family examined, ranging from twelve (or thirteen) in family PM2 (tetraploid interspecific hybrid: Fig. 4.13) to thirty eight

Table 4.3

Proportion of cross-compatible and cross-incompatible pollinations in each F_1 family

Family Species	Ploidy	Cross-compatible (%)	Cross-incompatible (%)
L. rigidum	2x		
B5x08		81.6	18.4
01x02		74.5	25.5
L3xB1		94.6	5.4
L. multiflorum	2x		
ED1		70.3	29.7
ED2		69.4	30.6
ED3		61.4	38.6
L. multiflorum	4x		
Lm3		65.7	34.3
Lm4		68.5	31.5
L. perenne	2x		
M1		93.0	7.0
L. perenne	4x		
Lp1		74.1	25.9
Lp2		88.8	11.2
L. perenne x *L. multiflorum* interspecific hybrid	4x		
PM1		70.9	29.1
PM2		76.2	23.8

Table 4.4

Proportions of paired pollination results for each F_1 family

Species	Ploidy	Family	+ ⟷ + (%)	- ⟷ - (%)	+ ⟷ - (%)	χ^2	p
L. rigidum	2x						
		B5x08	70.8	3.7	25.5	10.61	0.01-0.02
		01x02	54.2	4.2	41.7	42.05	<0.001
		L3xB1	91.9	0.8	7.3	25.81	<0.001
L. multiflorum	2x						
		ED1	48.5	8.6	42.9	43.65	<0.001
		ED2	52.1	14.6	33.3	17.89	<0.001
		ED3	41.4	20.0	38.6	38.72	<0.001
	4x	Lm3	44.7	11.5	43.8		
		Lm4	46.9	9.8	43.4		
L. perenne							
	2x	M1	87.5	1.6	10.9	18.19	<0.001
	4x	Lp1	58.3	16.7	25.0		
		Lp2	79.2	2.0	18.9		
L. perenne x L. multiflorum interspecific hybrid	4x						
		PM1	59.3	5.6	35.2		
		PM2	47.4	15.8	36.8		
Østerbye et al (1980)			68.8	13.5	17.7		

Table 4.4 contd.

Key:

+ ⟷ +	reciprocally compatible
- ⟷ -	reciprocally incompatible
+ ⟷ -	one way compatible
χ^2	comparison between paired results observed and those of Østerbye et al. (1980)
p	probability of obtaining χ^2 value

Table 4.5

Proportions of paired pollination results for each F_1 family, assuming reciprocal crosses to be identical

Family / Species	Ploidy	+ ⟷ + (%)	- ⟷ - (%)	+ ⟷ - (%)	χ^2	p
L. rigidum	2x					
B5x08		72.6	11.8	15.6	0.67	0.80-0.70
01x02		58.3	9.9	31.8	13.80	0.01-0.001
L3xB1		90.9	3.3	5.8	22.81	<0.001
L. multiflorum	2x					
ED1		58.9	17.7	23.4	4.57	0.20-0.10
ED2		66.1	27.1	6.8	20.52	<0.001
ED3		50.4	26.9	22.7	15.21	<0.001
Lm3	4x	48.1	18.1	33.7		
Lm4		52.7	15.7	31.6		
L. perenne						
M1	2x	87.7	1.6	10.7	18.45	<0.001
Lp1	4x	72.3	22.7	5.0		
Lp2		81.3	3.5	15.2		
L. perenne x L. multiflorum interspecific hybrid	4x					
PM1		62.0	24.6	13.4		
PM2		72.7	11.4	15.9		
Østerbye et al. (1980)		68.8	13.5	17.7		

Table 4.5 contd.

Key:		
	+ ⟷ +	reciprocally compatible
	- ⟷ -	reciprocally incompatible
	+ ⟷ -	one way compatible
	χ^2	comparison between paired results, calculated assuming reciprocal crosses to be identical and those of Østerbye et al. (1980)
	p	probability of obtaining χ^2 value

in Lp2 (tetraploid perennial: Fig. 4.11(a)).

In addition to recording the proportion of crosses which were compatible or incompatible each F_1 family was analysed on the pairing system devised by Østerbye et al. (1980). The pairs of crosses were scored as: reciprocally compatible, reciprocally incompatible and one way compatible. The results of this type of analysis carried out on the thirteen F_1 families are presented in Tables 4.4 and 4.5 (extended results: Appendix 1, Table 2). Østerbye et al. (1980) proposed a novel system for the genetic control of a gametophytic self-incompatibility system by three loci, where one of the original loci had been duplicated (for example S'S''Z). The system was suggested as a possible explanation of results obtained from studies on the genetic control of self-incompatibility in L. perenne (Spoor, 1976) and L. multiflorum (Hay, 1978). This approach allows for the phenomenon of reciprocally incompatible plants having different genotypes, and a high level of one way compatibility in a diallel cross, as only two alleles (one S and one Z) have to be matched in pollen and pistil to produce an incompatible reaction. For one specific cross the proportions of the paired reactions expected in a F_1 diallel were given by Østerbye et al. (1980) as:

68.8% reciprocally compatible;

13.5% reciprocally incompatible; and

17.7% one way compatible. The results of the paired analyses are presented in Tables 4.4 and 4.5, as proportions of the three types of paired reaction. For each diploid F_1 family, the results obtained have been compared to the theoretical expectations of Østerbye et al.

(1980) using a χ^2 test. The results of the χ^2 tests presented in Table 4.4 show that the seven diploid families are unlikely to be of the type proposed by Østerbye et al. (1980). As the diallels contained blanks where crosses had not been performed, a theoretical exercise was carried out filling in blanks on the assumption that crosses would be reciprocally identical (Table 4.5). This reduced the frequency of one way compatible crosses and increased the frequency of reciprocally compatible and reciprocally incompatible crosses (Table 4.4 ; Table 4.5). As a result, two diploid families B5x08 (annual: Fig. 4.1) and ED1 (biennial: Fig. 4.4) exhibited similar proportions of the three types of paired reaction to the theoretical family of Østerbye et al. (1980). In addition, there was a higher level of cross-compatible reactions in diploid than in tetraploid F_1 families and a corresponding lower level of cross-incompatible and one way compatible reactions in diploid than in tetraploid F_1 families (Table 4.4). Previous studies on induced tetraploids of Secale cereale (Lundqvist, 1957) and Festuca pratensis (Lundqvist, 1962b) demonstrated that the gametophytic self-incompatibility system was retained, but modified so that only one S and one Z allele carried in the pollen had to be matched to produce an incompatible reaction. This resulted in a reduction in the frequency of cross-compatibility and an increase in the frequency of one way compatible reactions. In addition, diallels between progeny produced as a result of crossing tetraploid S. cereale or F. pratensis resulted in plants which were reciprocally incompatible, but had different mating types.

In order to investigate further the self-incompatibility genotypes present, consideration was given to the mating reaction of each individual plant within a diallel. In a gametophytic

self-incompatibility system controlled by two or more genes, individuals heterozygous at most or all of the S-loci could be expected to be successful as pollen donors. In contrast, individuals homozygous for most of the S-loci would be expected to be successful as pollen receivers. In order to quantify this characteristic, the percentage of cross-incompatible reactions (out of the total crosses performed for that individual) were calculated for each plant in every F_1 family. For each individual, separate calculations were made representing percentage cross-incompatibility as a male, and as a female, and the difference obtained (Table 4.6). The sign of the difference between the two values would give an indication of the success of a plant as a male or female, such that negative values would indicate plants heterozygous at most S-loci and positive values plants with more homozygous S-loci. (Values calculated refer only to S-loci, not to the overall heterozygosity of a plant, which was not known). When either the male or female array of an individuals reactions was incomplete, the calculated difference (Table 4.6) did not always give an accurate indication of the plants reactions in the diallel. This resulted in three types of error which are represented on Table 4.6 by the following symbols:

(i) * an asterisk indicates that the sign of the value is wrong. For example, plant 25 in the family 01x02 has a heterozygosity value of -15.0, but was more successful as a female than as a male, and a positive number would have been expected;

(ii) ‡ indicates the number was expected to be zero as the plant was equally successful as a male and as a female;

Table 4.6

Heterozygosity value of S-loci calculated for plants in each F_1 family

Family: / Plant	B5x08	01x02	L3xB1	ED1	ED2	ED3
1	-15.0		- 5.3			-18.2
2	-23.5		- 4.0			
3	- 1.3 ‡		-11.5	33.9		- 0
4		10.4			-13.1	
5	1.5 *		0	-19.5	1.4	
6	6.2	0.2 *	0.5 ‡		- 3.3 *	20.7
7	-17.3		- 8.8		-25.0 *	-16.7
8	20.8	-27.8	- 4.2		- 2.8 *	
9			0.4 ‡	11.4	- 7.1	- 0
10	25.0	-11.8	4.0		2.9 *	
11	3.3 ‡		4.7		6.7	
12	17.5		- 4.5			
13	10.4	7.5	- 6.7	4.7	-40.0	-69.2 ‡
14	25.0	14.3	6.6	10.0	13.6	- 7.6
15		30.1		-28.9	53.8	16.7
16	- 7.0		0		- 3.8	
17	18.8		0.3 ‡		-35.7	
18	0		3.8	- 1.5	-15.0 *	35.7
19	8.3	-15.8	0	-32.9		
20			12.5	15.6		- 7.7
21	-12.3	- 2.5 ‡	0.3 ‡	7.2	- 3.1	
22	20.9	-10.6	0	- 8.0		13.3
23		-41.7	9.1		10.9	
24		7.6	1.1 ‡	7.0	42.9	3.8 ‡
25		-15.0 *	5.0			
26	-46.7	6.8	- 4.1	7.8 *	5.7	
27	9.4	0	9.5	-28.9		1.7
28	1.8	18.9	-11.1	2.1 *	49.5	35.7
29		-14.1 ‡	0	12.5	10.2	-25.5
30	5.6	21.3		- 9.1	- 7.5 ‡	
31	- 6.2	-20.0		17.9	- 4.2	-40.0
32		9.7		19.1		
33		14.8		10.0	-27.5	
34	9.5	- 8.7		-23.3		- 9.8 ‡
35		13.1		-13.8 *		
36		0		0.6		
37		3.5			- 2.4 ‡	
38	-24.9			- 0	-20.5	
39				3.3	6.7 *	
40				-14.4	30.4	

Key: B5x08), 01x02), L3xB1) L. rigidum, diploid

ED1), ED2), ED3) L. multiflorum, diploid

For explanation of symbols see text.

Table 4.6 contd.

Heterozygosity value of S-loci calculated for plants in each F_1 family

Family: Plant	Lm3	Lm4	M1	Lp1	Lp2	PM1	PM2
1		- 3.9			-19.9		
2		0			-10.6		
3	-29.9 *	10.2			9.8		
4			- 3.6	-25.5	16.3		
5	17.2	- 1.0 *	10.9	0	6.4		8.3 *
6	- 6.7	10.3		22.2 ‡	7.4		- 8.9
7	- 3.3 *	- 1.5	-18.2	23.1	- 7.3		
8	- 9.7	- 8.7			- 9.7	-21.5	
9	- 3.7			0	6.3	5.0	
10		3.8	0	0	3.3	20.0 *	33.3
11	3.7		-13.6	+ 0	0.3 ‡	37.5	
12		13.5	9.1	-16.7	- 0.3 ‡	-31.8	
13			0	32.9	- 2.2		0
14	3.1	- 4.1			-14.5	- 3.8 *	-41.7
15		5.2 *		-42.9	15.2		
16	-11.4	-16.1	0	0	10.4	-15.3	-35.7
17			4.5		- 5.0	-16.7 ‡	
18	10.1	- 2.7			- 3.0	22.1	20.8
19	9.9		-13.4	-30.0	5.7	25.5	
20		4.2	0		- 6.8	- 6.3	
21					13.9		16.7
22	11.2	18.6		26.9 *	-11.7		20.0
23	8.9	5.5	- 4.6	-50.0	- 4.4	-16.7	
24		- 5.0	- 4.6	-13.3 ‡			33.3
25	15.3	- 7.1 ‡	0	-10.2 *	- 7.4	7.8 ‡	
26		-12.0	- 4.5		- 6.3	11.9	
27	-19.2			-16.7	7.1	14.3 *	- 8.3 ‡
28			9.1		- 3.0	4.2 ‡	0
29	- 7.5	23.3	- 9.1	10.0	5.4		0
30	- 7.7	- 6.9	9.1	4.2	18.1		
31	-13.4	- 3.2		0	- 3.1	-13.5	
32	1.6 ‡	-18.1	4.5	-25.0	21.9		
33		3.9	9.1		-11.0	20.8	
34			9.1	10.0	- 2.7	-25.0 ‡	
35	12.6	9.6 ‡	4.5	28.6	-11.5		
36	28.6	- 3.5	4.5	10.3	- 2.8	-18.6	
37	-16.5				6.1		
38	- 9.5	12.6		-12.5			
39					3.0		
40	- 1.4	-10.2			1.7		

Key: Lm3), Lm4) L. multiflorum, tetraploid

M1 L. perenne, diploid

Lp1), Lp2) L. perenne, tetraploid

PM1), PM2) L. perenne x L. multiflorum interspecific hybrid, tetraploid

For explanation of symbols see text.

(iii) +0 or -0 indicates that the difference between the two percentage cross-incompatibilities calculated should not have been zero and the sign before the zero shows whether the plant was more successful as a male (-0) or as a female (+0). In total, 52 of the 198 plants studied had misleading heterozygosity values.

By selecting plants in each F_1 family which had a high negative number and those which had a correspondingly high positive number (highest and lowest heterozygosity values) a small array could be created. Within each section of every small diallel for each F_1 family the percentage cross-incompatibility was calculated. Thus, it was expected that plants with heterozygous S-loci would be compatible as pollinators with all other plants; and that plants with homozygous S-loci would be generally incompatible as pollinators of heterozygous plants but compatible as pollinators of homozygous plants (Fig. 4.14(a)). A number was selected for a cut-off point between plants having high and low negative heterozygosity values to ensure that all the plants with heterozygous S-loci were cross-compatible (Table 4.7). As expected, there was a high frequency of cross-incompatible reactions in the homozygous ♂ x heterozygous ♀ category (Table 4.7: Fig. 4.14(a)). However, contrary to expectations there was a low frequency of cross-incompatible reactions in the heterozygous ♂ x homozygous ♀ category (Table 4.7) in five of the thirteen F_1 families. However, in the four diploid F_1 families this was the result of plants with heterozygous and homozygous S-loci being reciprocally incompatible.

The calculated heterozygosity values were used to rearrange

Fig. 4.14

(a) Expected perecentage cross-incompatibility between plants with heterozygous and homozygous S-loci

♀ \ ♂	heterozygous	homozygous
heterozygous	0	high
homozygous	0	low

(b) Expected areas of cross-incompatibility and cross-compatibility when plants are arranged by heterozygosity value

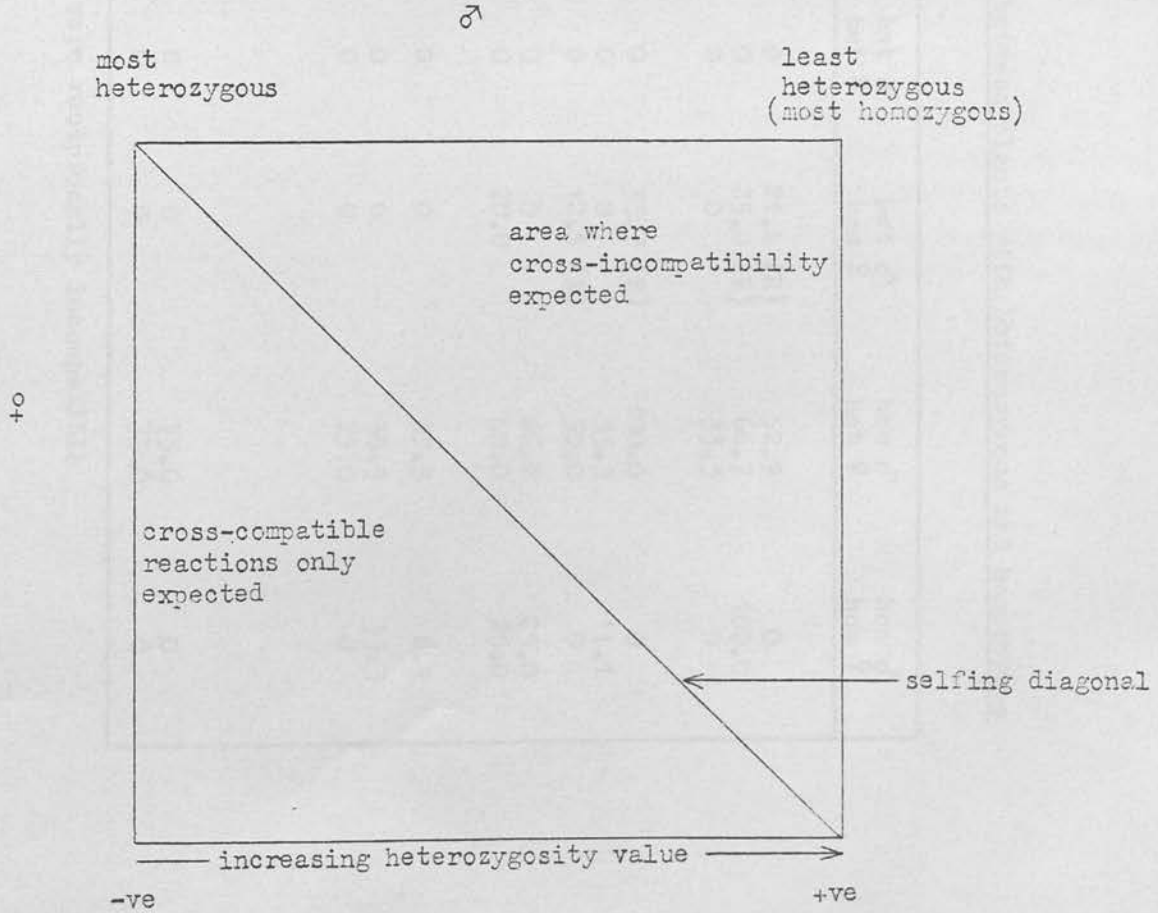


Table 4.7

Proportion of incompatible reactions observed in crosses between plants with heterozygous and homozygous S-loci, for each F_1 family

Family Species	Ploidy	Heterozygosity Value	Number of plants	het ♂ het ♀	het ♂ hom ♀	hom ♂ het ♀	hom ♂ hom ♀
L. rigidum	2x						
B5x08		$\geq \pm$ 15	11	0	21.4 (R)	52.9	0
01x02		$\geq \pm$ 16	5	0	25.0 (R)	66.7	100.0
L3xB1		$\geq \pm$ 9	5	0	0	33.3	0
L. multiflorum	2x						
ED1		$\geq \pm$ 20	5	0	33.3 (R)	100.0	0
ED2		$\geq \pm$ 21	8	0	0	33.3	11.1
ED3		$\geq \pm$ 20	6	0	12.5 (R)	50.0	0
Lm3	4x	$\geq \pm$ 13	6	0	0	66.7	25.0
Lm4		$\geq \pm$ 13	5	0	20.0	40.0	25.0
L. perenne							
M1	2x	$\geq \pm$ 9	10	0	0	33.3	6.9
Lp1	4x	$\geq \pm$ 20	10	0	0	38.5	33.3
Lp2		$\geq \pm$ 14	6	0	0	25.0	0
L. perenne x L. multiflorum interspecific hybrid	4x						
PM1		$\geq \pm$ 21	6	0	0	75.0	0
PM2		$\geq \pm$ 9	7	0	0	75.0	0

Key: (R) plants with homozygous and heterozygous S-loci were reciprocally incompatible
het plants with mainly heterozygous S-loci
hom plants with mainly homozygous S-loci

results of the diallel crosses by placing the plants in decreasing order of heterozygosity from left to right. The rearranged diallel for family B5x08 (diploid annual: Fig. 4.1) is given in Fig. 4.15. Cross-incompatible reactions would only be expected to occur between plants where the male had fewer heterozygous S-loci than the female (Fig. 4.14(b)). The rearranged diallels of diploid F_1 families did not conform to the expected pattern (Fig. 4.14(b) cf Fig. 4.15).

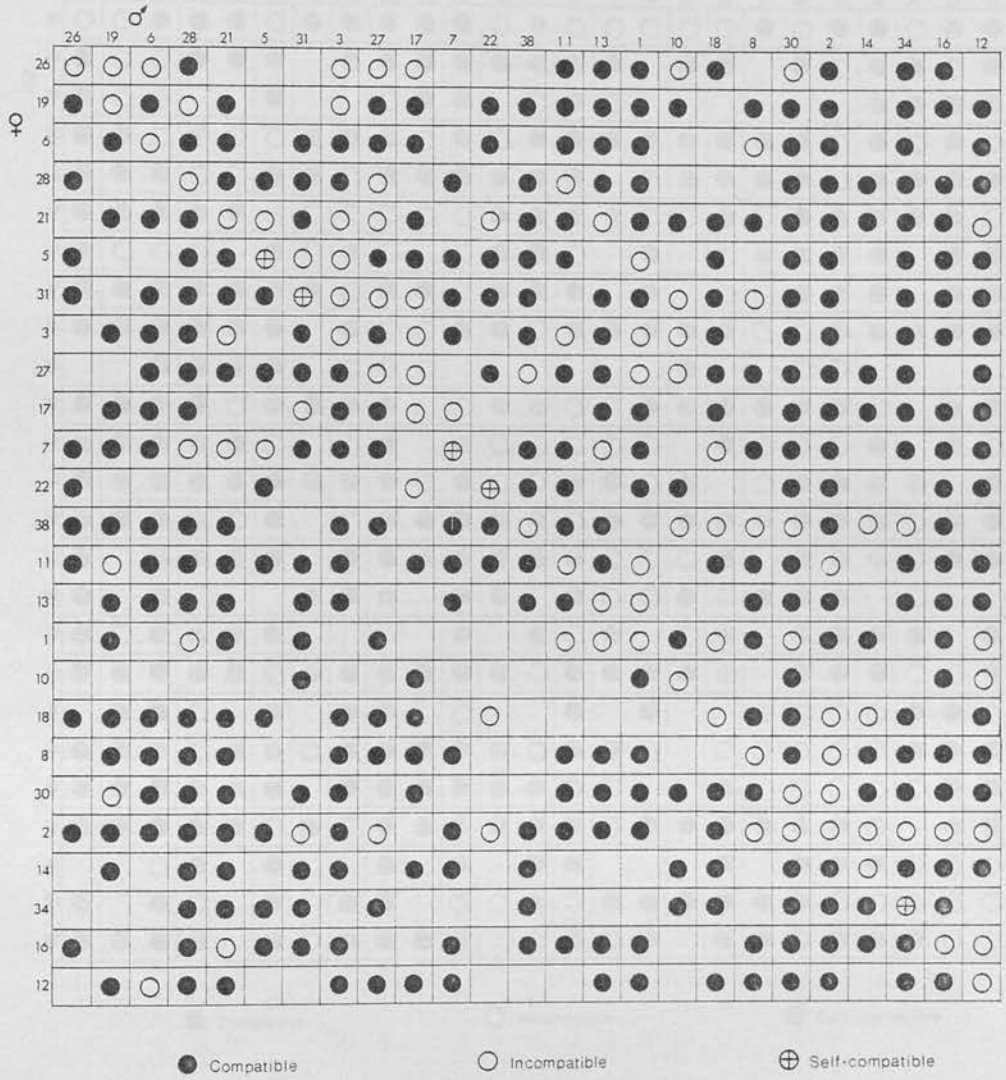


Fig. 4.1

F_1 family B5x08: results of crosses between twenty-five plants produced by a controlled cross between two diploid L. rigidum plants from different populations (B and 0).

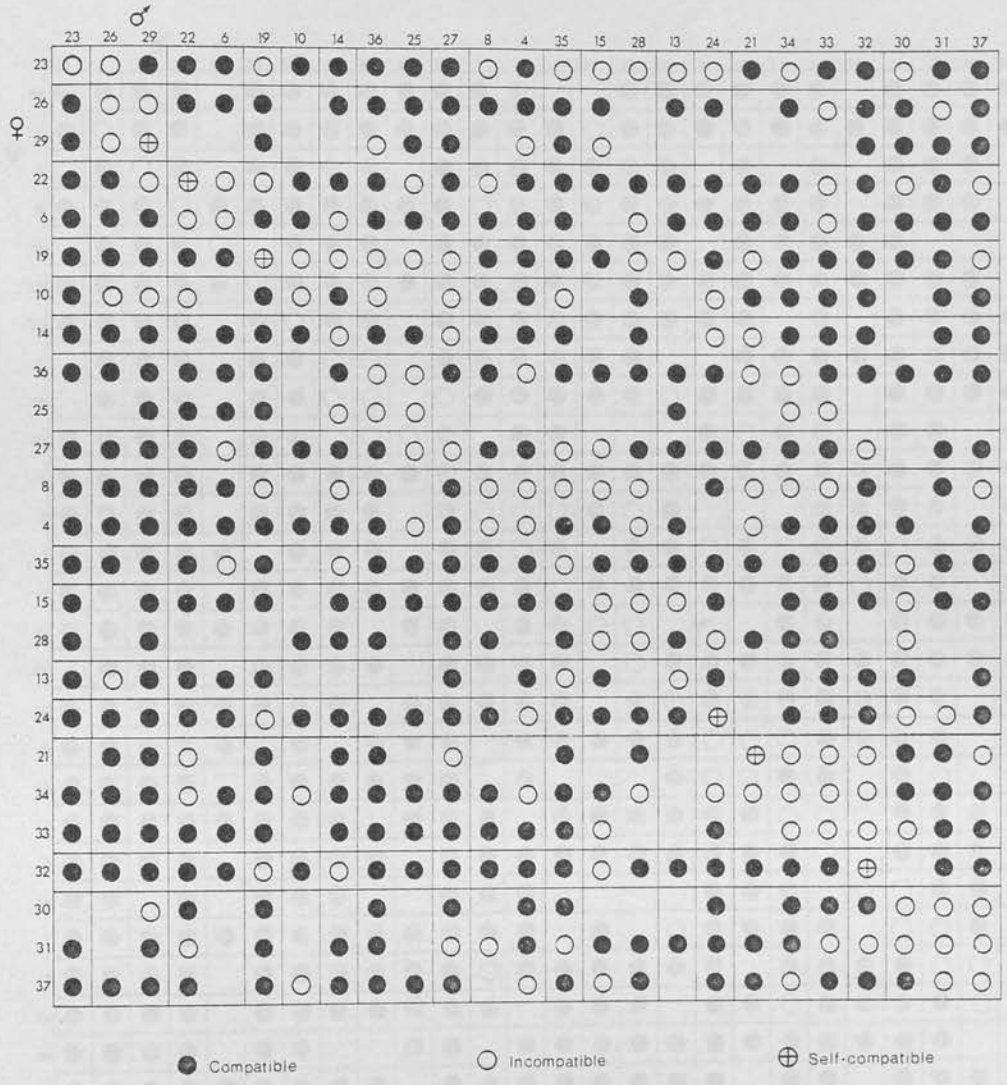


Fig. 4.2

F_1 family 01x02: results of crosses between twenty-five plants produced by a controlled cross between two diploid L. rigidum plants from the same population (0).

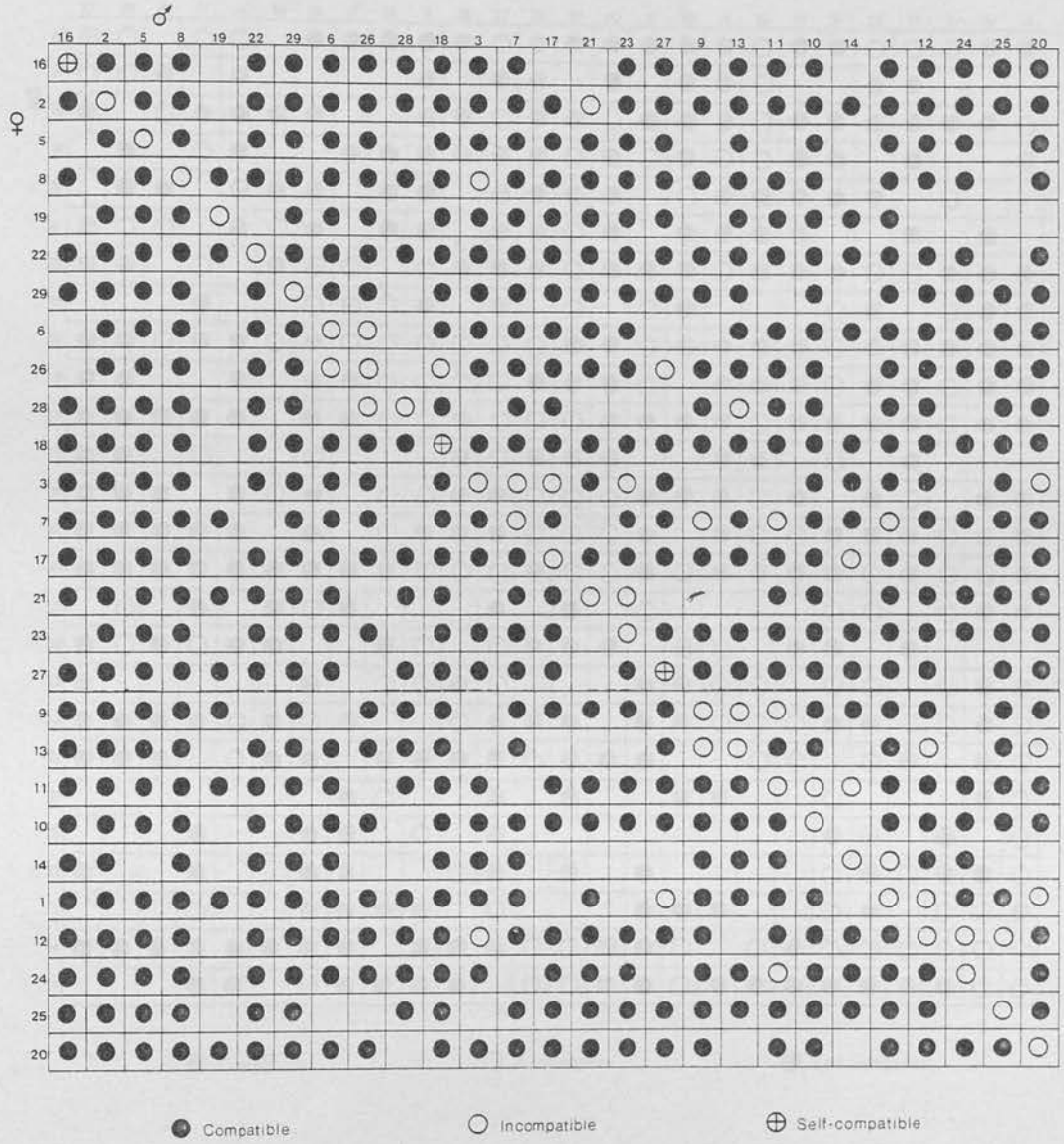


Fig. 4.3

F_1 family L3xB1: results of crosses between twenty-seven plants produced by a controlled cross between two diploid L. rigidum plants from different populations (L and B).

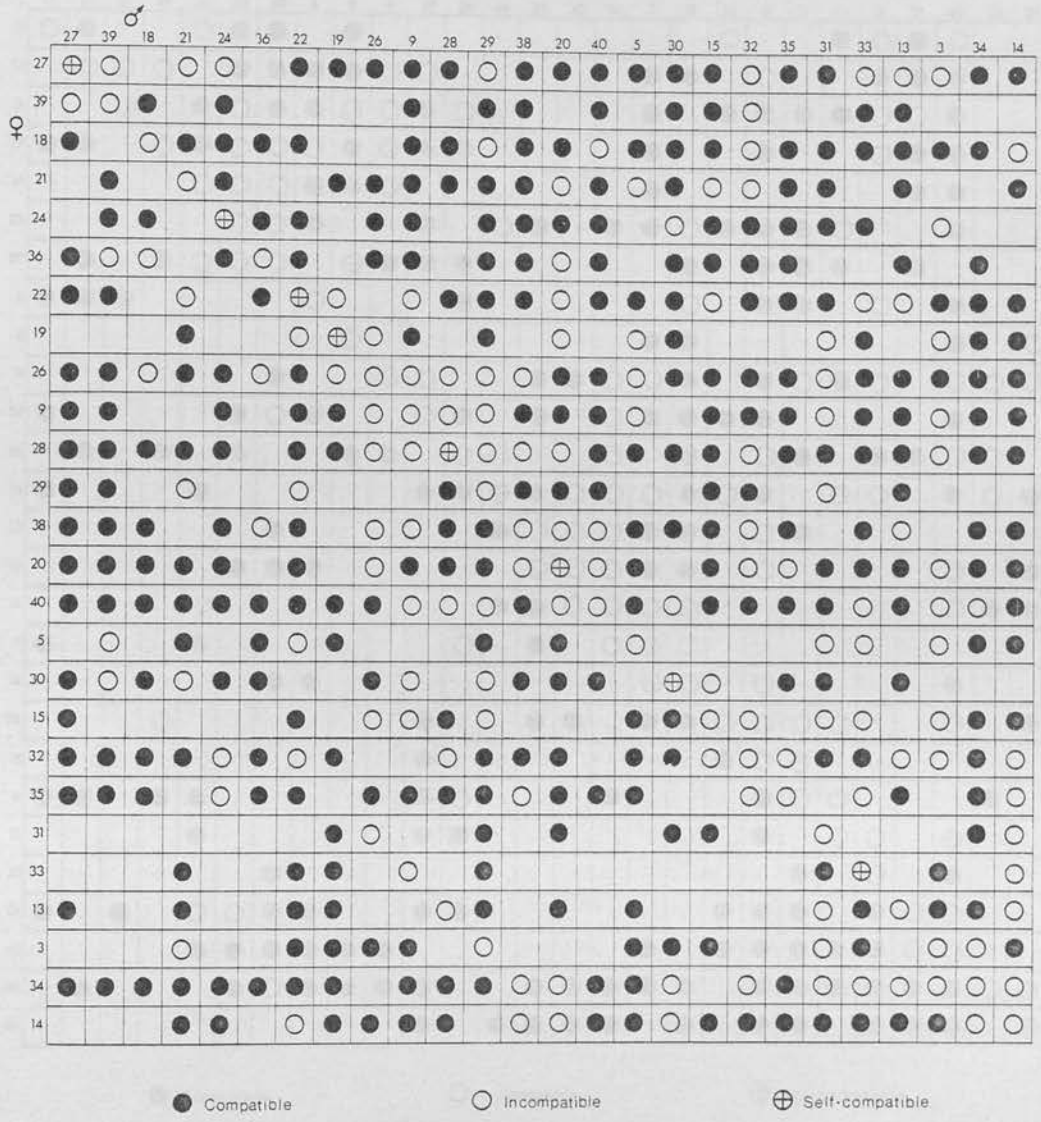


Fig. 4.4

F_1 family ED1: results of crosses between twenty-six plants produced by a controlled cross between two diploid L. multiflorum plants from different commercial varieties (S22 and Sceemster).

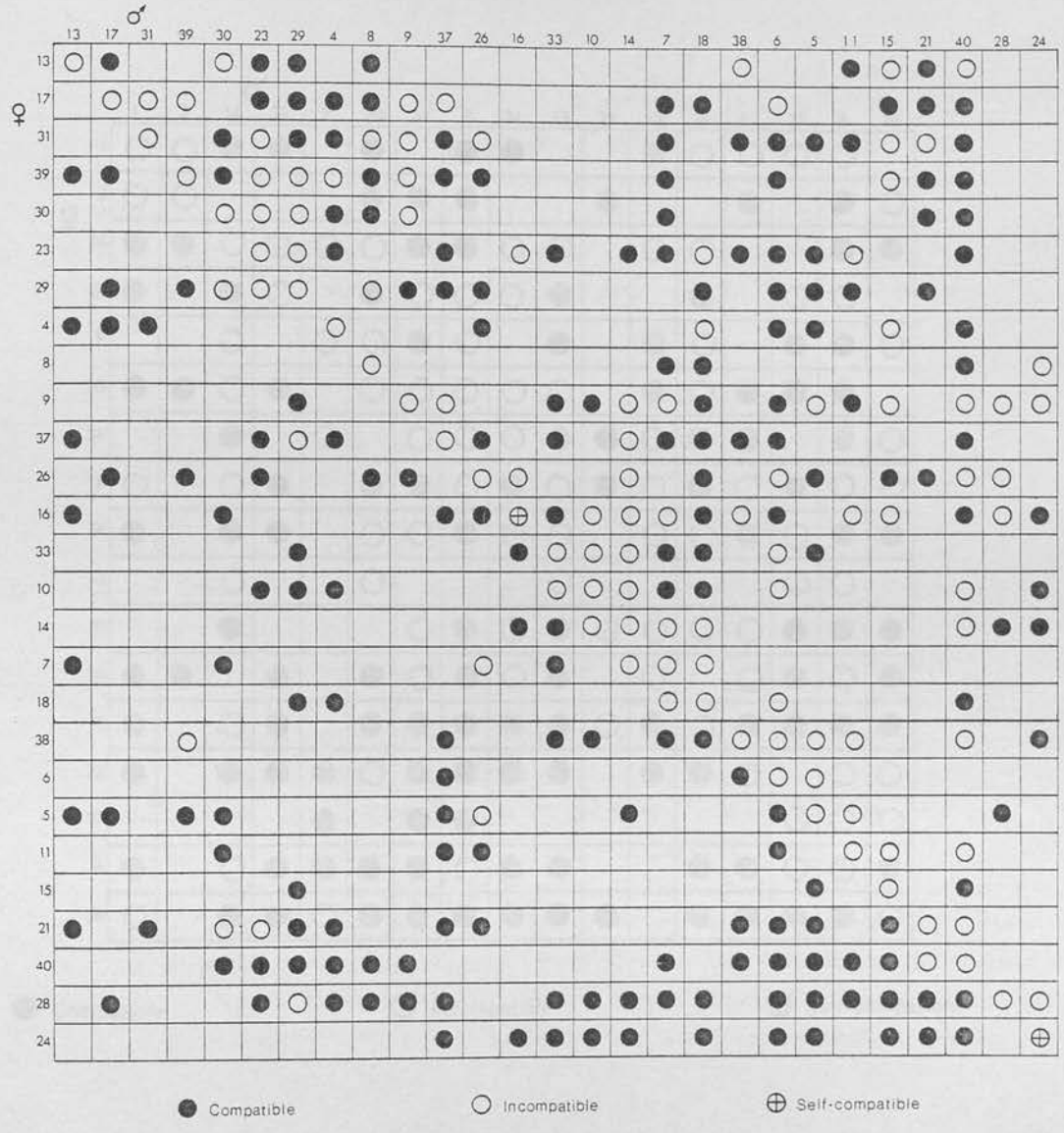


Fig. 4.5

F_1 family ED2: results of crosses between twenty-seven plants produced by a controlled cross between two diploid L. multiflorum plants from different commercial varieties (Sceemster and Combita).

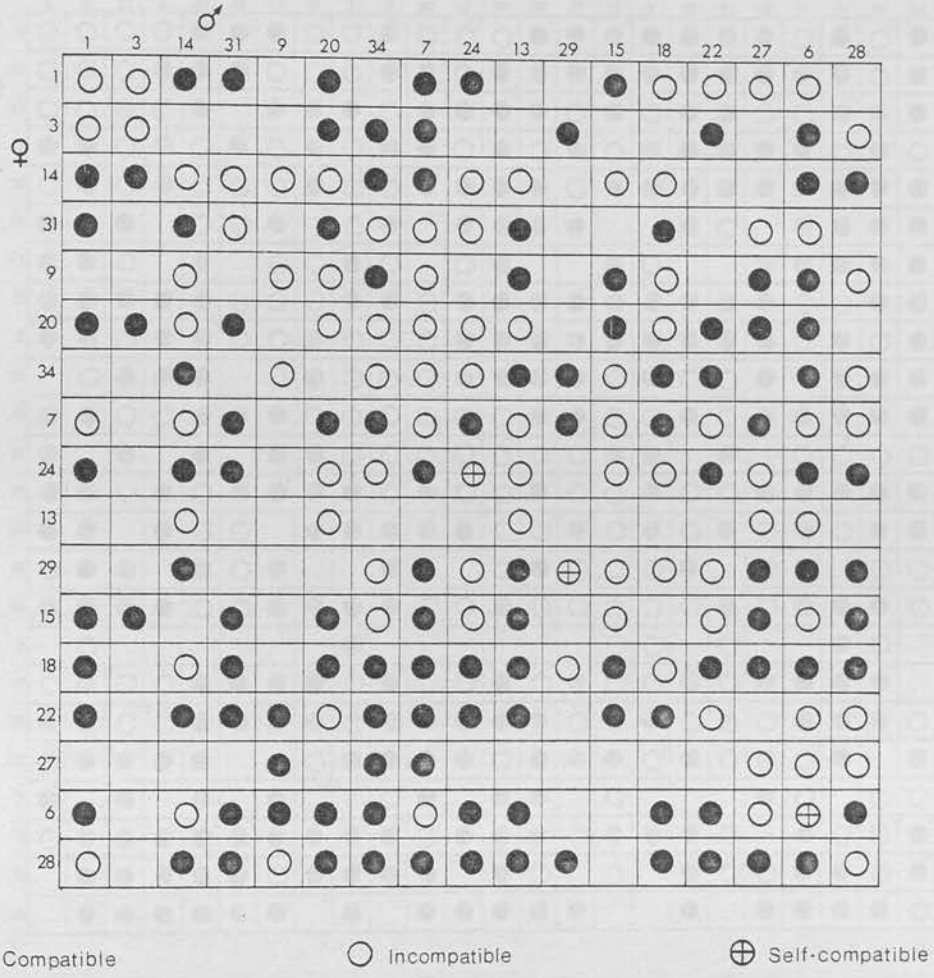


Fig. 4.6

F_1 family ED3: results of crosses between seventeen plants produced by a controlled cross between two diploid L. multiflorum plants from different commercial varieties (S22 and Combita).

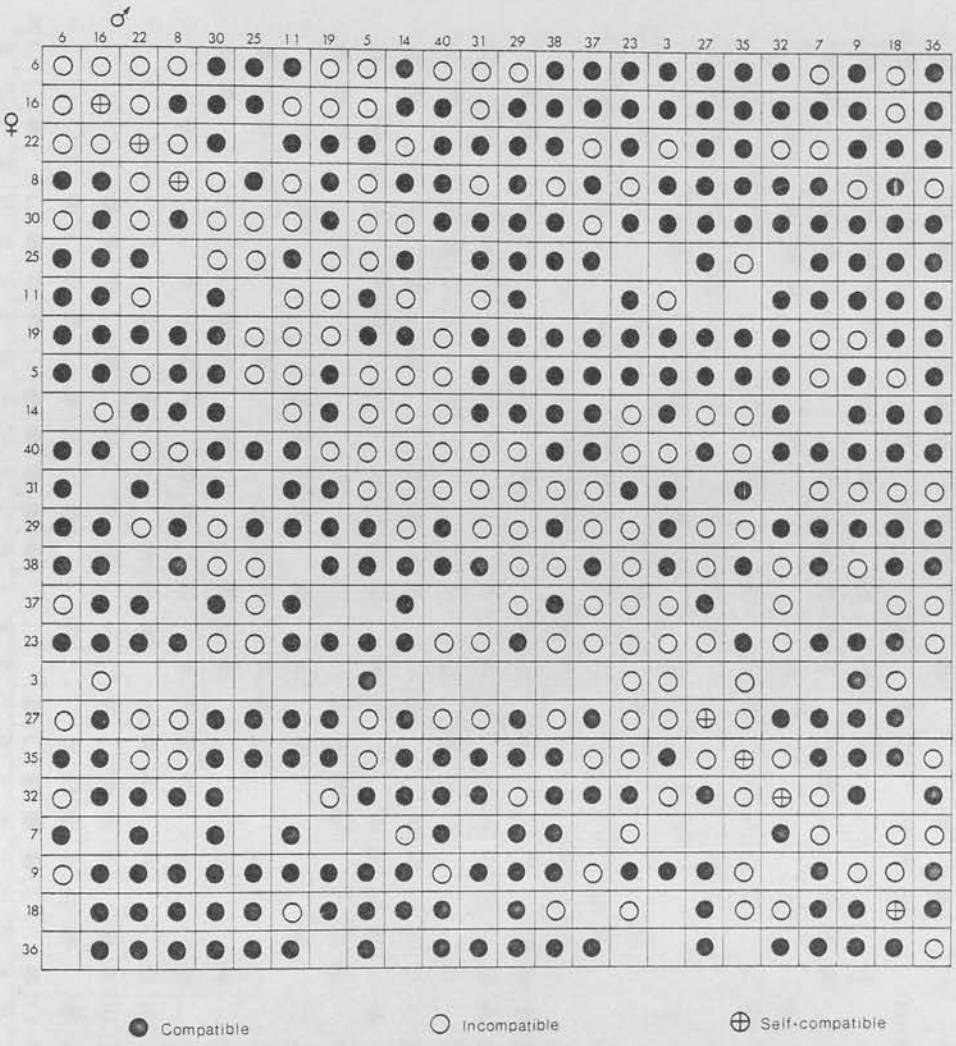


Fig. 4.7

F_1 family Lm3: results of crosses between twenty-four plants produced by a controlled cross between two tetraploid L. multiflorum plants from the same experimental line (B6 1419).

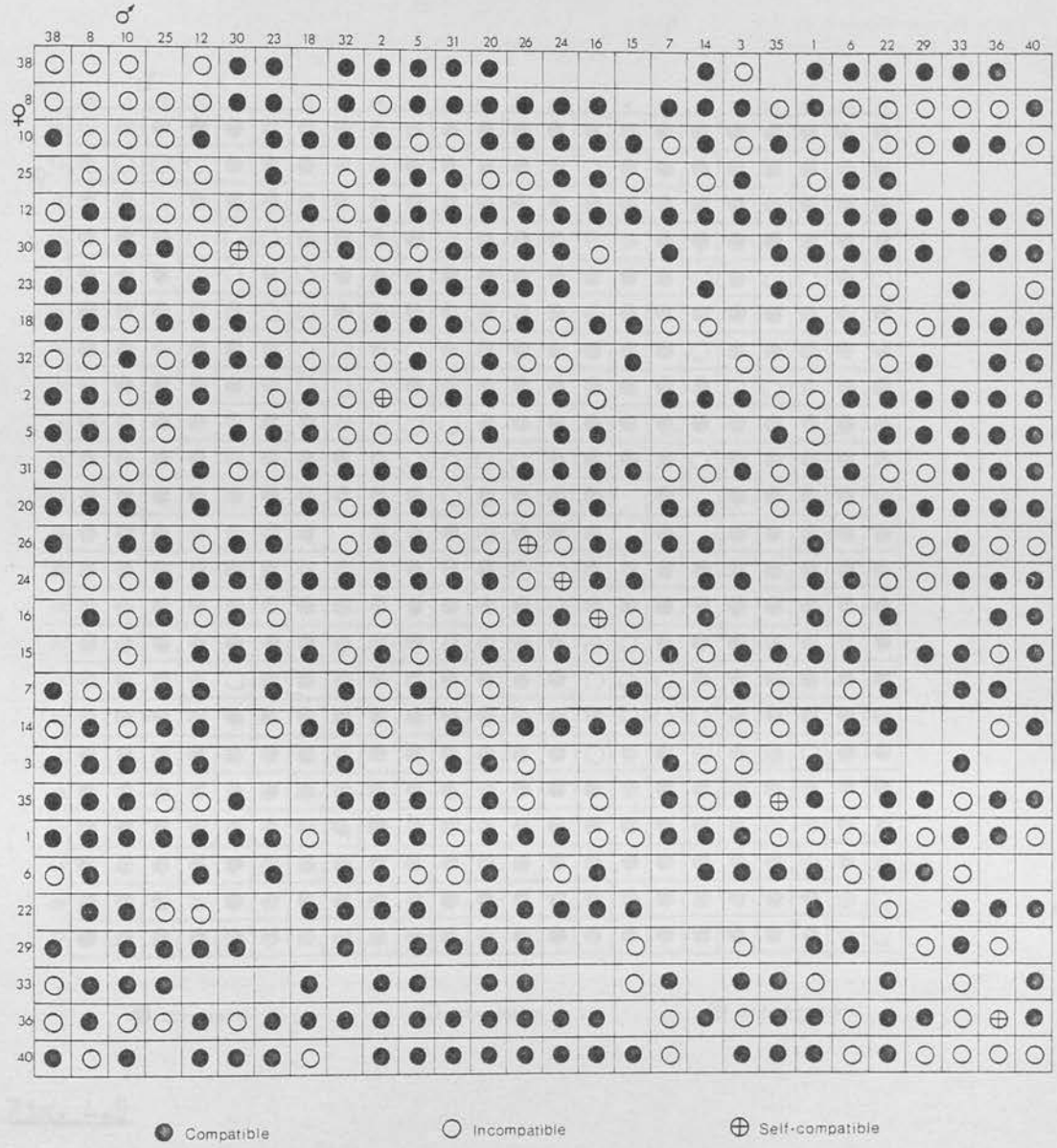


Fig. 4.8

F_1 family Lm4: results of crosses between twenty-eight plants produced by a controlled cross between two tetraploid L. multiflorum plants from the same experimental line (B6 1419).

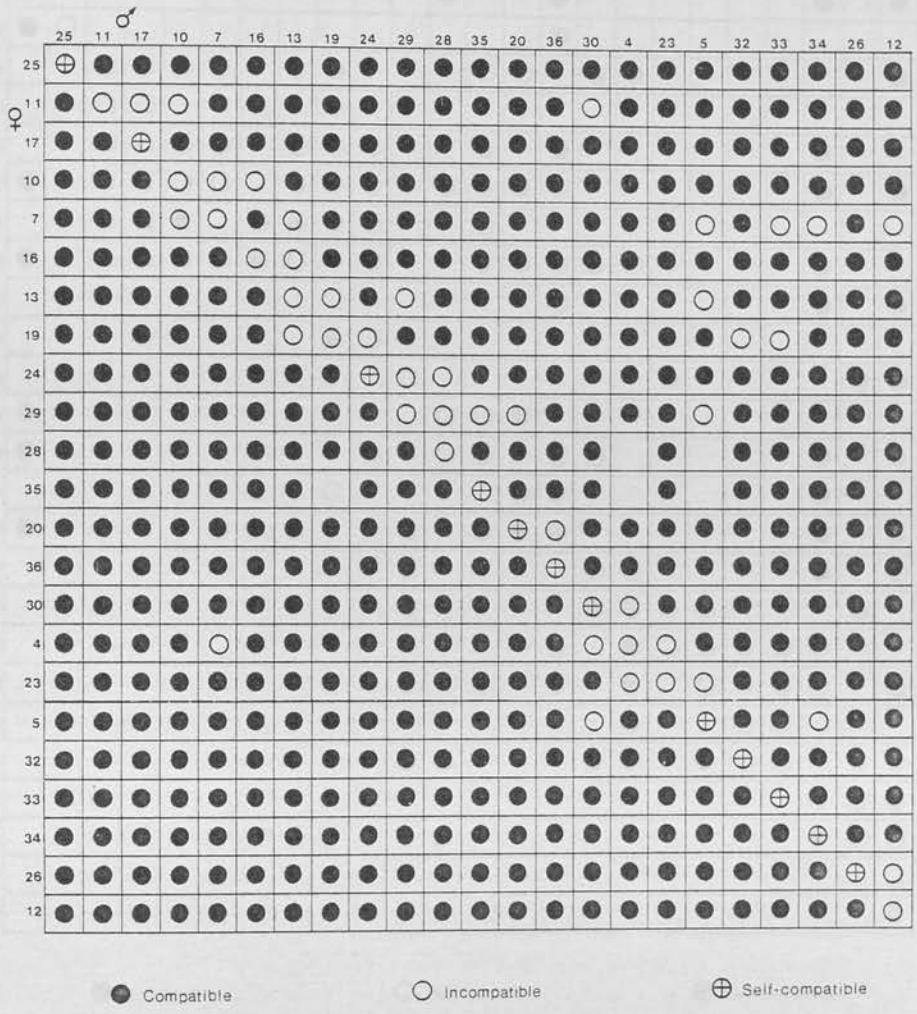


Fig. 4.9

F_1 family M1: results of crosses between twenty-three plants produced by a controlled cross between two diploid L. perenne plants from different experimental lines (Ba 8979 and Ba 8981).

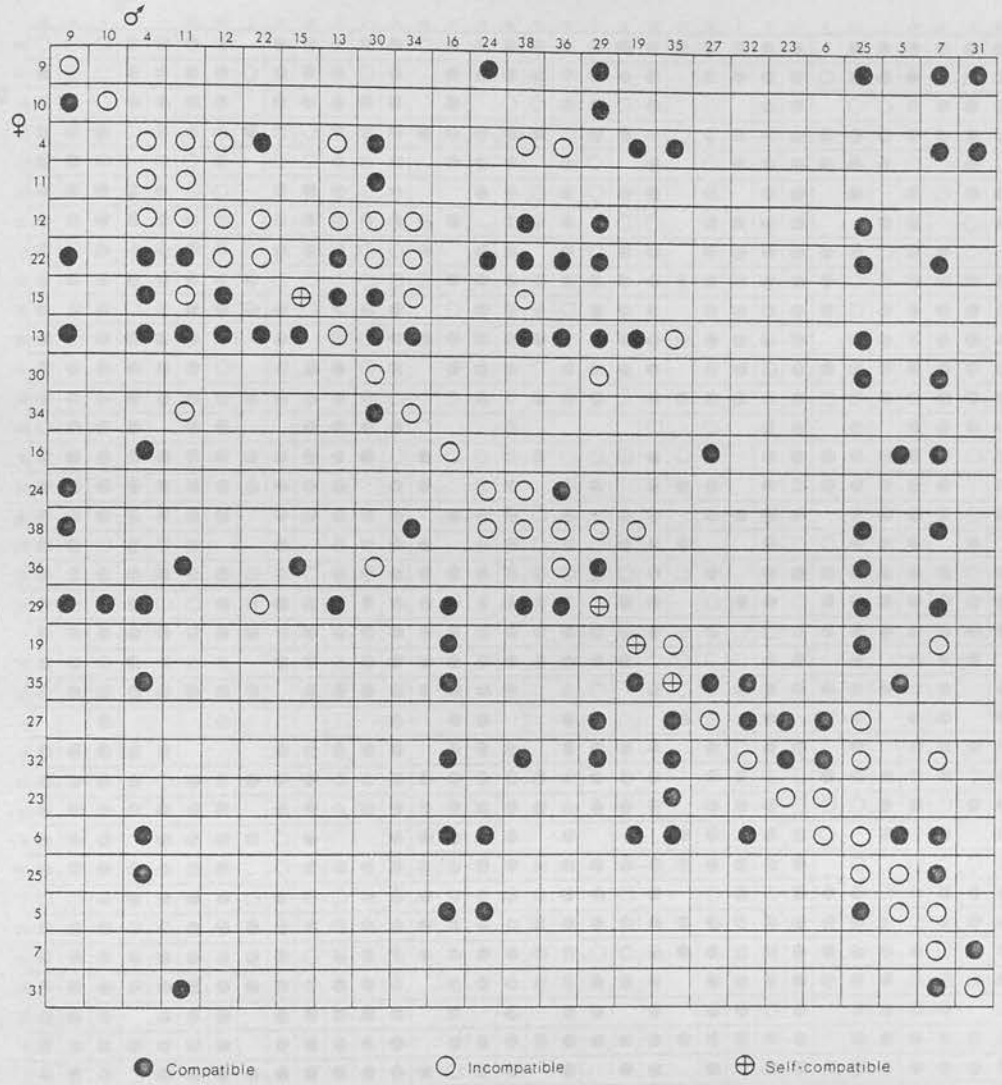


Fig. 4.10

F_1 family Lp1: results of crosses between twenty-five plants produced by a controlled cross between two tetraploid L. perenne plants from different experimental lines (Ba 8982 and Ba 8983).

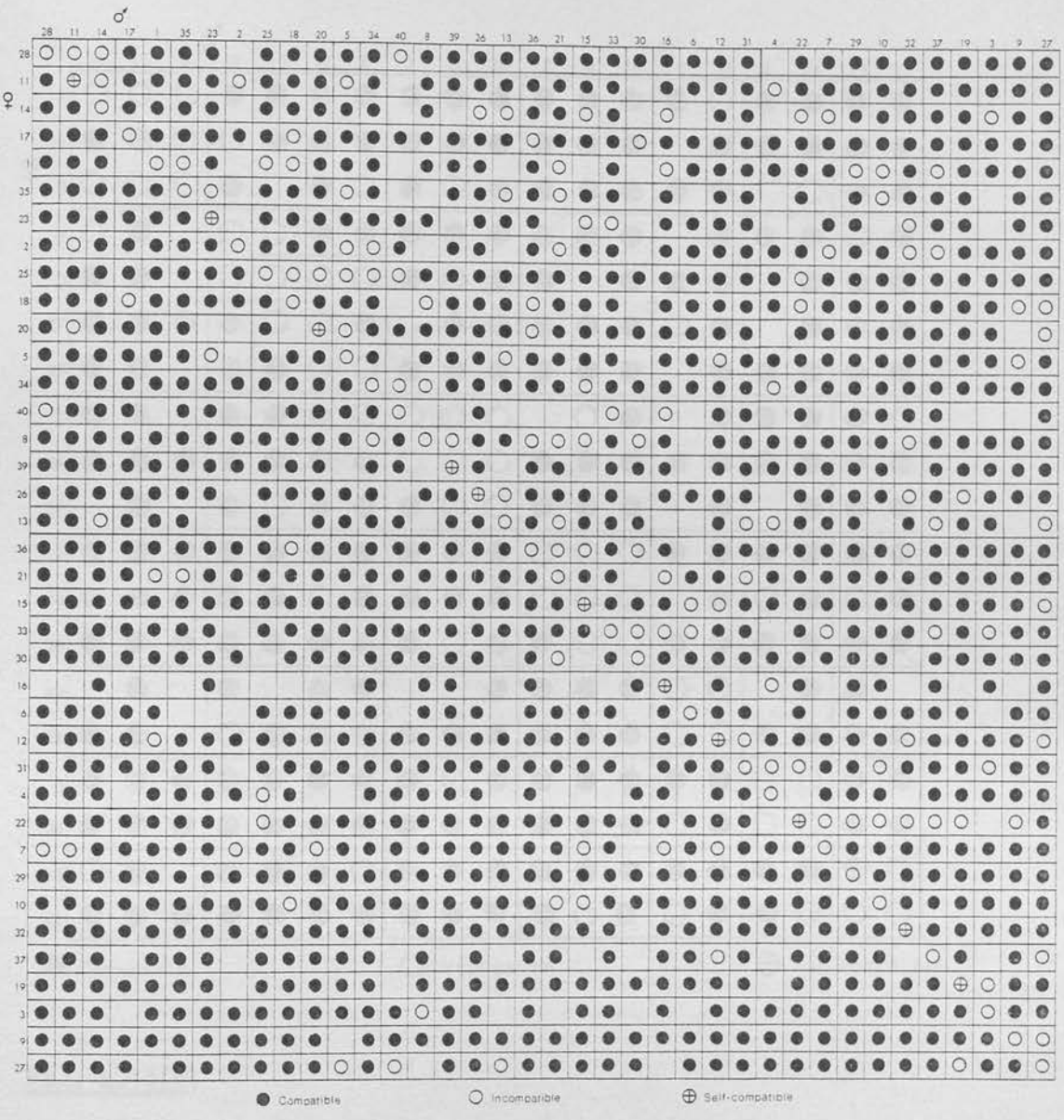


Fig. 4.11(a)

F_1 family Lp2: results of crosses between thirty-eight plants produced by a controlled cross between two tetraploid L. perenne plants from the same experimental line (Ba 8982).

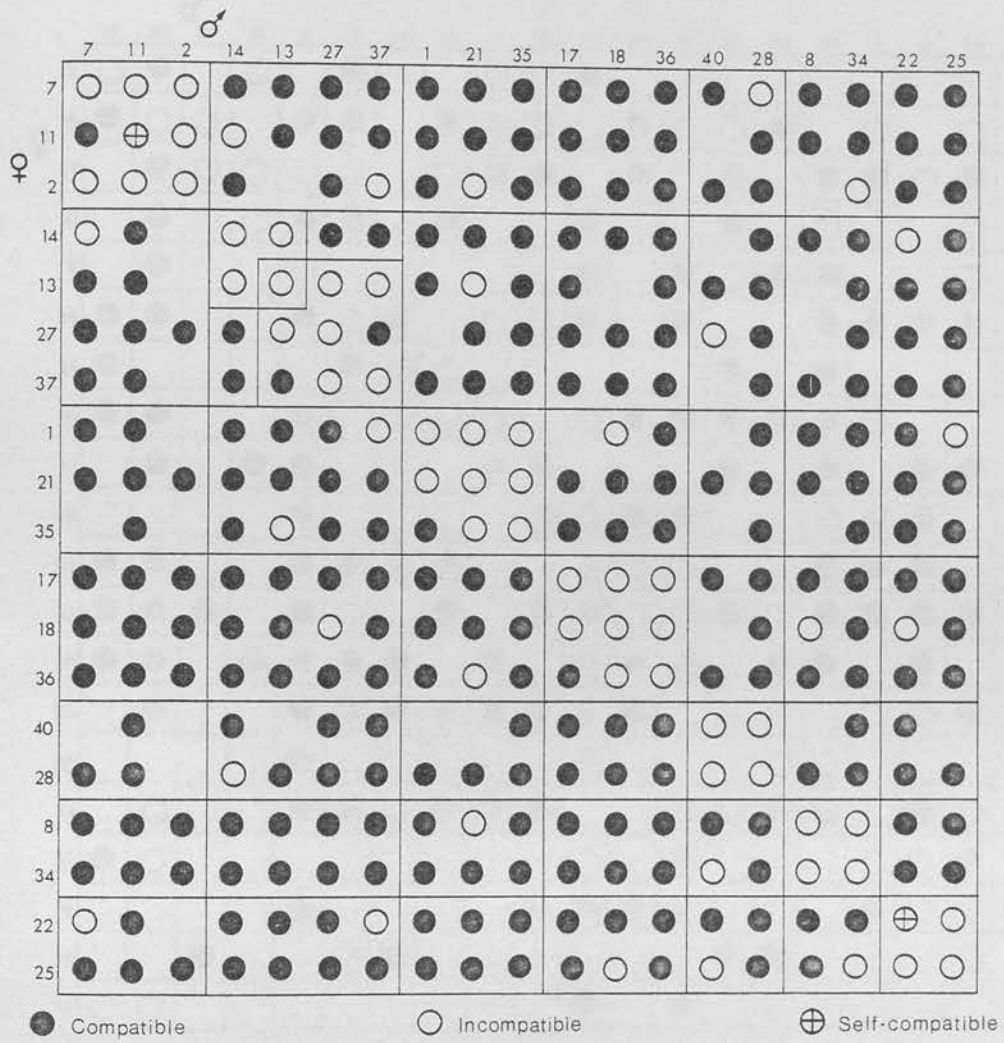


Fig. 4.11(b)

F_1 family Lp2: results of crosses between nineteen of the thirty-eight tetraploid *L. perenne* plants arranged to show intra-incompatible groups.

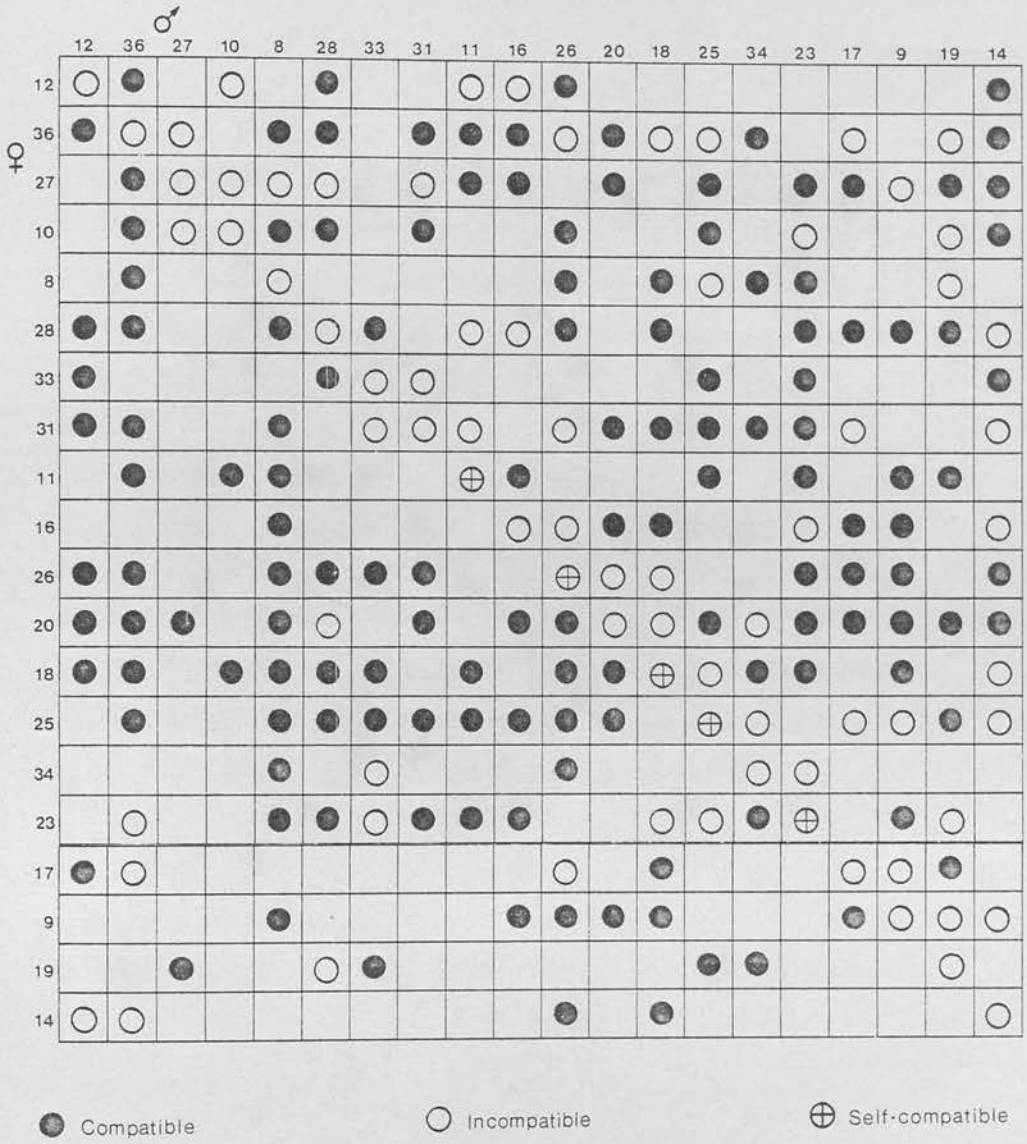


Fig. 4.12

F_1 family PM1: results of crosses between twenty plants produced by a controlled cross between tetraploid L. perenne (Ba 8982) and L. multiflorum (Bb 1419) plants.

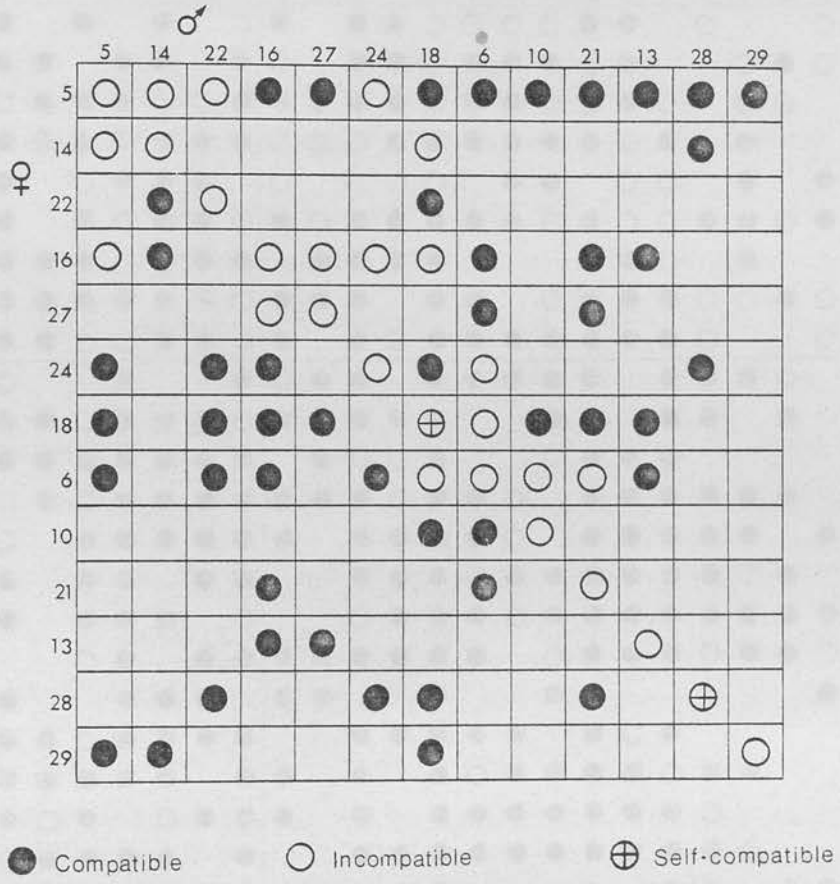


Fig. 4.13

F_1 family PM2: results of crosses between thirteen plants produced by a controlled cross between tetraploid L. perenne (Ba 8982) and L. multiflorum (Bb 1419) plants.

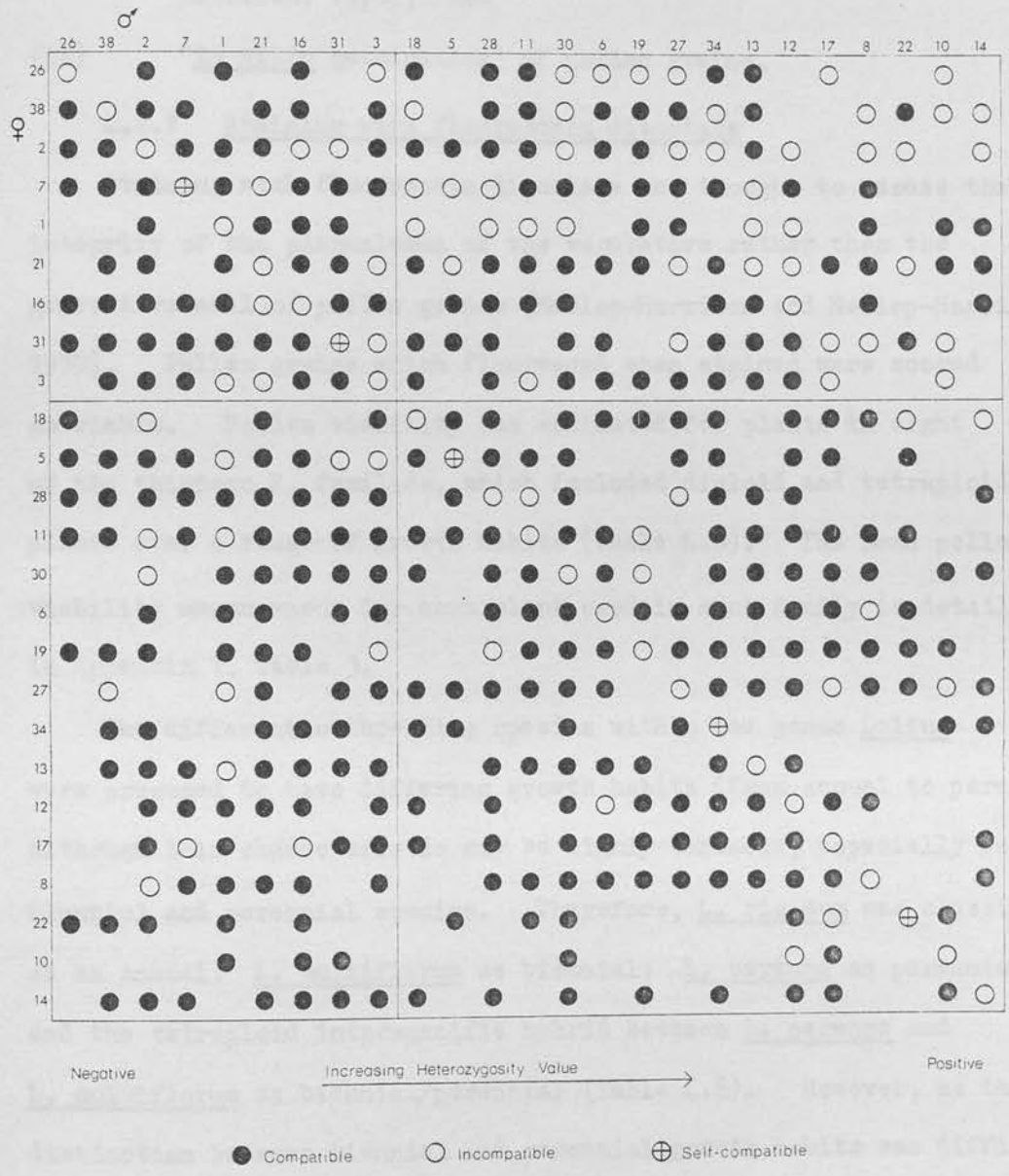


Fig. 4.15

F_1 family B5x08: results of crosses between twenty-five diploid *L. rigidum* plants, arranged in increasing order of heterozygosity value from left (most negative) to right (most positive).

4.2 Pollen viability

Two methods of estimating pollen viability were examined:

(i) fluorescein diacetate stain (Heslop-Harrison and Heslop-Harrison, 1970); and

(ii) "*in vitro* germination" of pollen grains.

4.2.1 Staining with fluorescein diacetate

Staining with fluorescein diacetate was thought to assess the integrity of the plasmalemma of the vegetative rather than the generative cell of pollen grains (Heslop-Harrison and Heslop-Harrison, 1970). Pollen grains which fluoresced when stained were scored as viable. Pollen viability was estimated for plants in eight of the thirteen F_1 families, which included diploid and tetraploid plants over a range of growth habits (Table 4.8). The mean pollen viability measurement for each plant used in each family is detailed in Appendix 1, Table 3.

The different outbreeding species within the genus *Lolium* were presumed to have differing growth habits (from annual to perennial), although this characteristic may be highly variable, especially for biennial and perennial species. Therefore, *L. rigidum* was classified as an annual; *L. multiflorum* as biennial; *L. perenne* as perennial; and the tetraploid interspecific hybrid between *L. perenne* and *L. multiflorum* as biennial/perennial (Table 4.8). However, as the distinction between biennial and perennial growth habits was difficult to assess (due to lack of time the true behaviour of the families studied was unknown) it was later decided to divide the families into annual and non-annual species. This resulted in three categories of families: diploid annuals, diploid non-annuals and tetraploid non-annuals (Table 4.8). The mean pollen viabilities for each

Table 4.8

Families used to estimate pollen viability by staining with fluorescein diacetate

			Chromosome number	
			Diploid	Tetraploid
Growth Habit	Annual		O1xO2 L3xB1	
	Non-annual	Biennial		Lm3 Lm4
		Hybrid (biennial x perennial)		PM1 PM2
		Perennial	M1	Lp2

family and for each category are given in Table 4.9.

By using the different categories outlined above, Analyses of Variance were carried out to test the following null hypotheses, that there was no difference in mean pollen viability between:

(i) diploid and tetraploid F_1 ryegrass families;

(ii) annual and non-annual F_1 ryegrass families;

(iii) diploid annual, diploid non-annual and tetraploid non-annual F_1 ryegrass families; and

(iv) diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families.

The results showed that there was no significant difference in mean pollen viability between diploid and tetraploid F_1 ryegrass families (Table 4.10(i)) and between annual and non-annual F_1 families (Table 4.10(ii)). However, when the F_1 families were divided into categories combining ploidy and growth habit there was a highly significant difference in mean pollen viability between diploid annual, diploid non-annual and tetraploid non-annual F_1 families (Table 4.10(iii)) and between the different diploid and tetraploid Lolium species (Table 4.10(iv)). The unbalanced experimental design resulted in too few degrees of freedom to allow for tests of interaction between the characteristics ploidy and growth habit.

4.2.2 Pollen germination in vitro

Most studies of pollen cultured in vitro have used the percentage of grains which germinated in a given time as an index of the viability of the pollen sample. However, in this study germination was infrequent when pollen grains were widely spaced out but abundant in aggregates of pollen grains. This phenomenon has been

Table 4.9

Mean pollen viability for eight F_1 families used to estimate pollen stainability

			Chromosome number			
			Diploid	Tetraploid		
Growth Habit		Annual	54.65, 60.19 } 57.53			
	Non-annual	Biennial		58.79, 58.41 } 58.59		
		Hybrid		51.26, 53.51 } 52.15	} 58.46	
		Perennial	65.77	64.29		

Table 4.10

Analyses of Variance of pollen viability, estimated by staining with fluorescein diacetate:

(i) between diploid and tetraploid F_1 ryegrass families

Item	df	MS	p
between ploidies	1	149.06	0.2-0.05
residual	168	73.96	

(ii) between annual and non-annual F_1 ryegrass families

Item	df	MS	p
between growth habits	1	127.25	0.2-0.05
residual	168	74.09	

(iii) between diploid annual, diploid non-annual and tetraploid non-annual F_1 ryegrass families

Item	df	MS	p
between categories	2	547.25	<0.001
residual	167	68.74	

(iv) between diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families

Item	df	MS	p
between categories	4	836.56	<0.001
residual	165	56.93	

Key: df degrees of freedom
MS mean square
p probability of obtaining variance ratio

termed the population effect (Brewbaker and Kwack, 1963). Despite these difficulties, it was decided that measuring pollen tube length achieved by germinated pollen grains *in vitro* would give an indication of the vigour of the pollen grains (where vigour is defined as the ability of a pollen grain to germinate and grow *in vitro*).

For each plate, pollen tube length was measured for about thirty representative tubes. The diameter of the pollen grain producing the tube was also recorded. Germination was rapid, usually within one to two minutes of pollen being placed on the medium, with the maximum length being reached after one hour at room temperature. Bursting of grains was observed either before germination or shortly after the appearance of a tube from the germ pore. After two hours, tubes which had been formed began to burst and degenerate, possibly as a result of taking up water from the medium. Consequently, tube length was measured approximately one hour after pollen was spread on the culture medium. Occasionally branched tubes were observed, and rarely two tubes per pollen grain. The proportion of abnormalities was low in all families and was not recorded.

Pollen germination was studied in both diploid and tetraploid F_1 families, which all had non-annual growth habits (Table 4.11).

4.2.2.1 Pollen grain diameter

The mean pollen grain diameter (μm) of each plant studied in each family is detailed in Appendix 1, Table 4. The mean pollen grain diameter of each family is illustrated in Fig. 4.16. A null hypothesis that pollen grains from diploid and tetraploid plants were the same size was tested using an Analysis of Variance (Table 4.12.i).

Table 4.11

Families used in pollen germination in vitro studies

			Chromosome number	
			Diploid	Tetraploid
Growth Habit	Non-annual	Biennial		Lm4
		Hybrid		PM1
		Perennial	M1	Lp2

The results indicated that there was a highly significant difference in the mean size of pollen grains from diploid and tetraploid plants. There was no difference between the mean pollen grain diameter of the diploid and dihaploid pollen grains from the three tetraploid families (Table 4.12.ii).

4.2.2.2 Pollen tube length

The mean pollen tube length produced by pollen grains *in vitro* for each plant studied is detailed in Appendix 1, Table 5. The mean pollen tube length for each family is illustrated in Fig. 4.17. Analyses of Variance were carried out to test the null hypothesis that the mean pollen tube length produced by pollen grains which germinated *in vitro* was the same for all four F_1 families. The results of the Analysis of Variance of pollen tube length between diploid and tetraploid F_1 ryegrass families showed that the difference in mean pollen tube length was significant at the 5% level (Table 4.13.i). However, from the histogram (Fig. 4.17) of mean pollen tube length for each F_1 family it can be seen that one of the tetraploid F_1 families, Lp2 (*L. perenne*) produced pollen tubes less than half the length of those produced by the other tetraploid families, Lm4 and PM1. The null hypothesis that there was no difference in mean pollen tube length between the three tetraploid F_1 families was disproved (Table 4.13.ii). The results indicated that the mean pollen tube lengths for the two perennial families, M1 (diploid) and Lp2 (tetraploid) were very similar (Table 4.13.iii; Fig. 4.17).

Despite the successful germination and growth *in vitro* of pollen from the above four F_1 families, disappointing results were obtained with two additional families; L3xB1 (diploid, annual) and PM2 (the second tetraploid interspecific hybrid family). Flowering in the

Table 4.12

Analyses of Variance of pollen grain diameter:

(i) between diploid and tetraploid F_1 ryegrass families

Item	df	MS	p
between ploidies	1	678.85	< 0.001
residual	39	13.05	

(ii) between tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 families

Item	df	MS	p
between families	2	1.62	> 0.20
residual	30	14.40	

Key: df degrees of freedom
MS mean square
p probability of obtaining variance ratio

Table 4.13

Analyses of Variance of pollen tube length:

(i) between diploid and tetraploid F_1 ryegrass families

Item	df	MS	p
between ploidies	1	126701.70	0.05-0.01
residual	39	19313.12	

(ii) between tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families

Item	df	MS	p
between families	2	164009.05	< 0.001
residual	30	8232.78	

(iii) between diploid perennial and tetraploid perennial F_1 ryegrass families

Item	df	MS	p
between ploidies	1	1.94	> 0.20
residual	17	10684.79	

Key: df degrees of freedom
MS mean square
p probability of obtaining variance ratio

family PM2 was generally poor, only thirteen out of thirty-eight plants flowered. Of five plants tested only two produced pollen which germinated in vitro (Table 4.14). The mean pollen grain diameter was similar to that of other tetraploid plants (Fig. 4.16) and the average pollen tube length was approximately the same as previously observed for the other interspecific hybrid family, PM1 (Fig. 4.17). Flowering in the diploid annual, L. rigidum, L3xB1 was excellent, with twenty-seven out of a maximum of twenty-nine plants being included in the diallel. Pollen from eleven plants in this family was tested for germination and growth in vitro: pollen grains from nine of these plants did not germinate and grow in vitro, while short tubes were produced by pollen grains from the remaining plants (Table 4.14). Again, average pollen grain diameter and tube length were similar to those of the other diploid family, M1 (Figs. 4.16 and 4.17).

Table 4.14

Mean pollen grain diameter (μm) and pollen tube length (μm) for plants from families PM2 and L3xB1

Family	Plant	Pollen grain diameter mean (μm)	SEM	Pollen tube length mean (μm)	SEM	Number of observations
PM2	14	46.35	2.19	163.41	39.41	17
	24	42.86	1.65	505.08	65.34	24
	mean	44.31	1.77	363.41	173.33	41
L3xB1	10	34.64	1.35	93.62	18.01	23
	17	33.60	0.66	104.11	15.30	24
	mean	34.11	0.52	98.98	5.27	47

Key: PM2 *L. perenne* x *L. multiflorum* tetraploid interspecific hybrid

L3xB1 *L. rigidum*, diploid

SEM standard error of mean

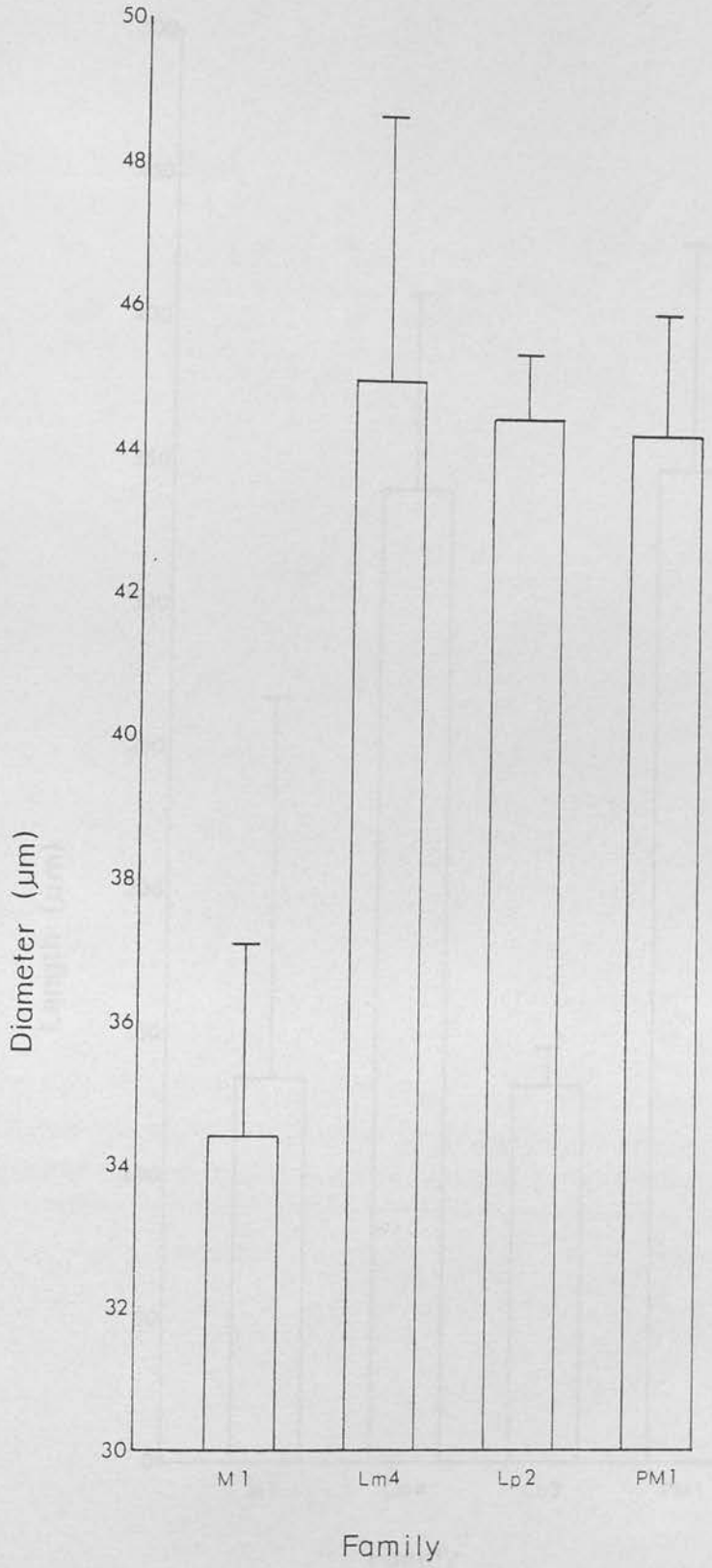


Fig. 4.16

Mean pollen grain diameter (µm) with 5% confidence limits for four F_1 families.

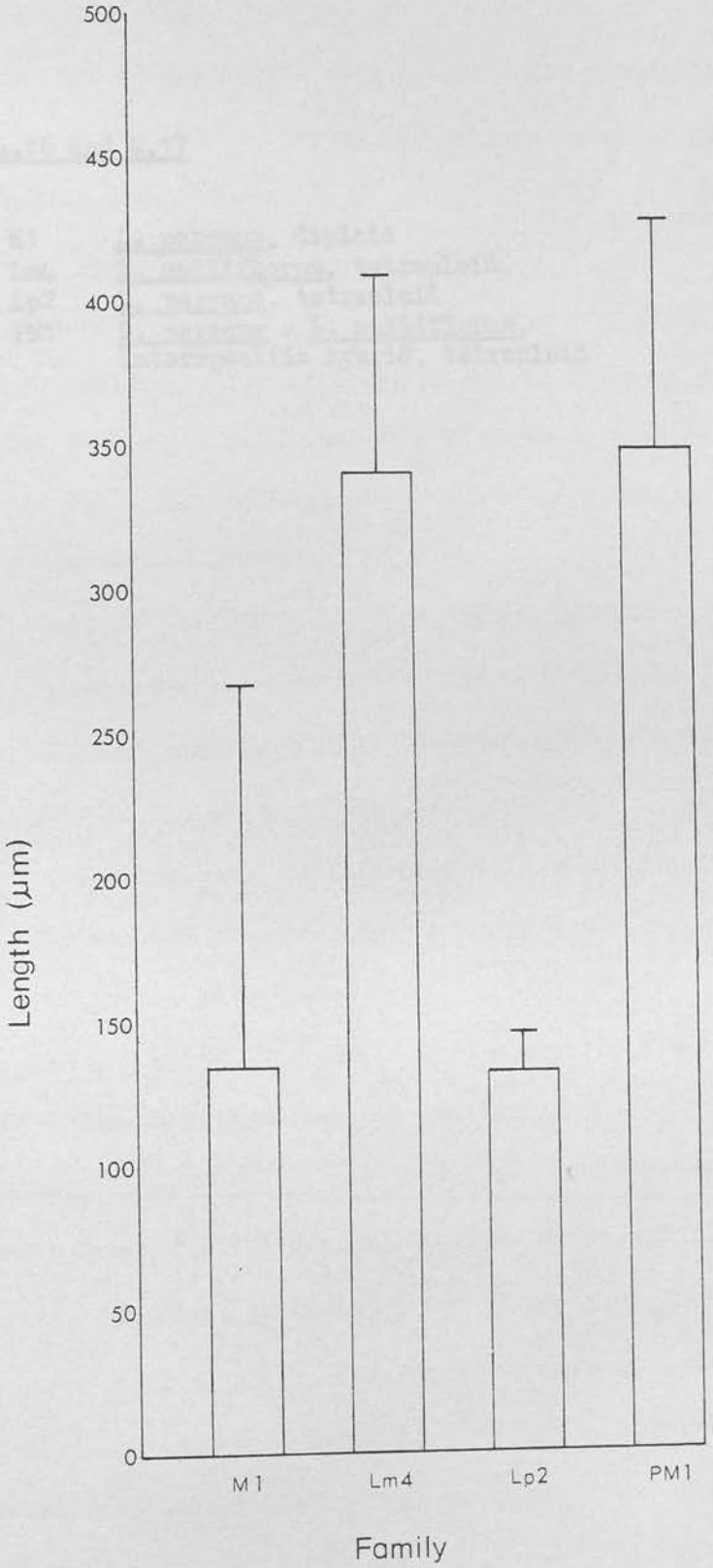


Fig. 4.17

Mean pollen tube length (µm) with 5% confidence limits for four F_1 families.

Figures 4.16 and 4.17

Key:

M1	*L. perenne*, diploid
Lm4	*L. multiflorum*, tetraploid
Lp2	*L. perenne*, tetraploid
PM1	*L. perenne* x *L. multiflorum*, interspecific hybrid, tetraploid

4.3 Anther and stigma length

Anther and stigma length were measured for plants in six F_1 families, both diploid and tetraploid, over a range of growth habits (Table 4.15).

4.3.1 Anther length

The mean anther length for each plant used is detailed in Appendix 1, Table 6. The mean anther length for each family is illustrated in Fig. 4.18. Analyses of Variance were carried out to test the following null hypotheses that there was no difference in mean anther length between:

(i) diploid and tetraploid F_1 ryegrass families;

(ii) annual and non-annual F_1 ryegrass families;

(iii) diploid annual, diploid non-annual and tetraploid non-annual F_1 ryegrass families; and

(iv) diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families.

The results showed that there were significant differences in mean anther length between diploid and tetraploid F_1 families at the 5% level, between annual and non-annual F_1 families at the 1% level and between diploid annual, diploid non-annual and tetraploid non-annual F_1 families at the 1% level (Table 4.16.i-iii). However, there were no differences in mean anther length between diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families (Table 4.16.iv).

Table 4.15

Families used for measurements of anther and stigma length

			Chromosome number	
			Diploid	Tetraploid
Growth Habit	Annual		L3xB1	
	Non-annual	Biennial		Lm4
		Hybrid		PM1 PM2
		Perennial	M1	Lp2

Table 4.16

Analyses of Variance of anther length:

(i) between diploid and tetraploid F_1 ryegrass families

Item	df	MS	p
between ploidies	1	0.78	0.05-0.01
residual	41	0.11	

(ii) between annual and non-annual F_1 ryegrass families

Item	df	MS	p
between growth habits	1	0.83	0.01-0.001
residual	41	0.11	

(iii) between diploid annual, diploid non-annual and tetraploid non-annual F_1 ryegrass families

Item	df	MS	p
between categories	2	0.50	0.05-0.01
residual	40	0.11	

(iv) between diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families

Item	df	MS	p
between categories	4	0.27	0.20-0.05
residual	38	0.11	

Key: df degrees of freedom
MS mean square
p probability of obtaining variance ratio

4.3.2 Stigma length

The mean stigma length for each plant from each family studied is detailed in Appendix 1, Table 7. The mean stigma length of each F_1 family is illustrated in Fig. 4.19.

Again, Analyses of Variance were carried out to test the following null hypotheses that there was no difference in mean stigma length between:

(i) diploid and tetraploid F_1 ryegrass families;

(ii) annual and non-annual F_1 ryegrass families;

(iii) diploid annual, diploid non-annual and tetraploid non-annual F_1 ryegrass families; and

(iv) diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families.

The results showed that there was no significant difference in mean stigma length between annual and non-annual F_1 families (Table 4.17.ii). However, there were hightly significant differences in mean stigma length (at the 0.1% level) between: diploid and tetraploid F_1 families; diploid annual, diploid non-annual, and tetraploid non-annual F_1 families; and diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families (Table 4.17.i,iii,iv).

Table 4.17

Analyses of Variance of stigma length:

(i) between diploid and tetraploid F_1 ryegrass families

Item	df	MS	p
between ploidies	1	7.29	<0.001
residual	41	0.30	

(ii) between annual and non-annual F_1 ryegrass families

Item	df	MS	p
between growth habits	1	1.00	0.20-0.05
residual	41	0.46	

(iii) between diploid annual, diploid non-annual and tetraploid non-annual F_1 ryegrass families

Item	df	MS	p
between categories	2	3.97	<0.001
residual	40	0.29	

(iv) between diploid annual, diploid perennial, tetraploid biennial, tetraploid perennial and tetraploid interspecific hybrid F_1 ryegrass families

Item	df	MS	p
between categories	4	2.47	<0.001
residual	38	0.26	

Key: df degrees of freedom
MS mean square
p probability of obtaining variance ratio

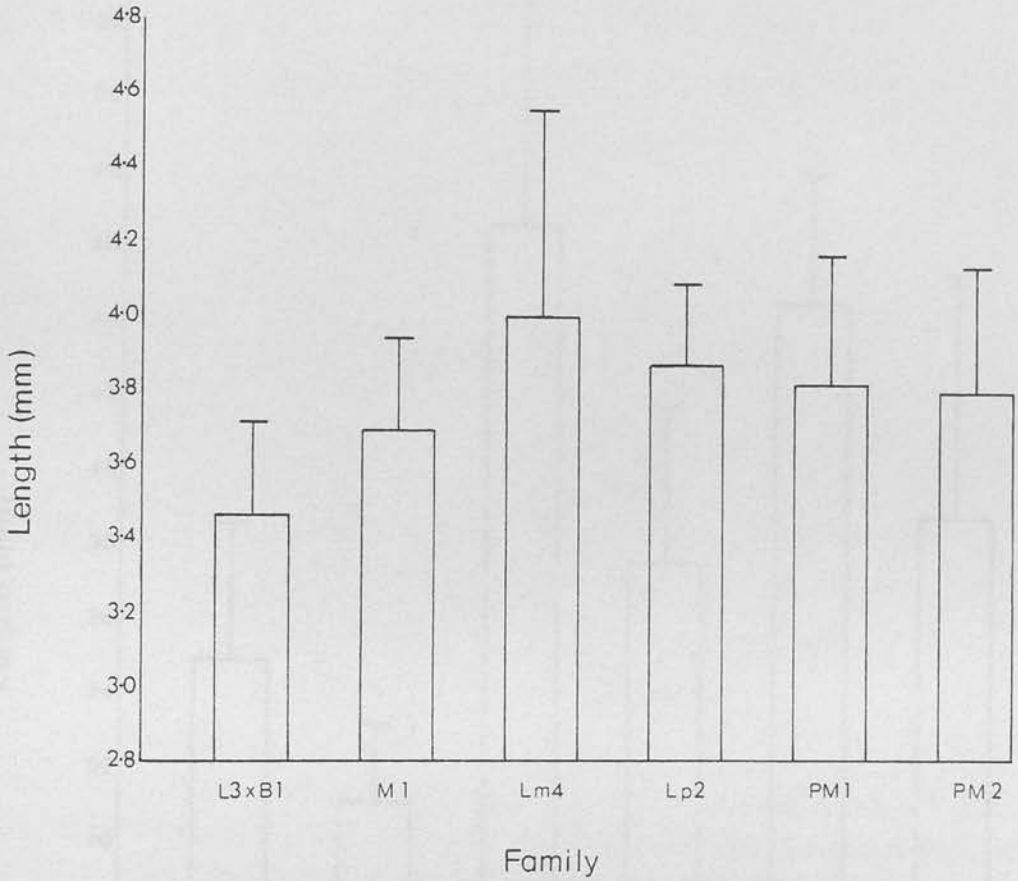


Fig. 4.18

Mean anther length (mm) with 5% confidence limits for six F_1 families.

Key:

L3xB1	L. rigidum, diploid
M1	L. perenne, diploid
Lm4	L. multiflorum, tetraploid
Lp2	L. perenne, tetraploid
PM1)	L. perenne x L. multiflorum,
PM2)	interspecific hybrid, tetraploid

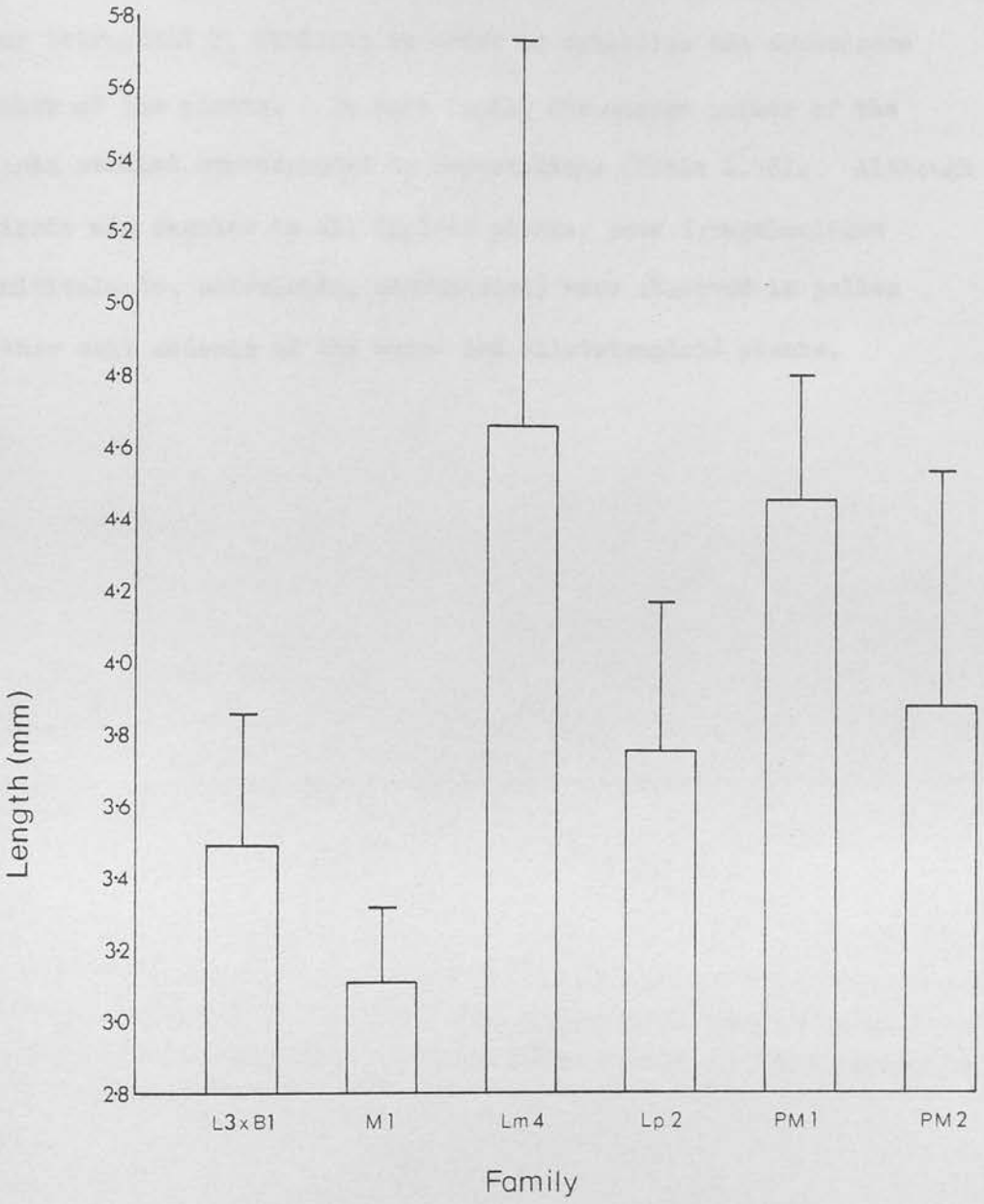


Fig. 4.19

Mean stigma length (mm) with 5% confidence limits for six F_1 families.

Key:
- L3xB1 — L. rigidum, diploid
- M1 — L. perenne, diploid
- Lm4 — L. multiflorum, tetraploid
- Lp2 — L. perenne, tetraploid
- PM1, PM2 — L. perenne x L. multiflorum, interspecific hybrid, tetraploid

4.4 Cytology

Pollen mother cell meiosis was studied in two diploid and four tetraploid F_1 families in order to establish the chromosome number of the plants. In each family chromosome number of the plants studied corresponded to expectations (Table 4.18). Although meiosis was regular in all diploid plants, some irregularities (multivalents, univalents, micronuclei) were observed in pollen mother cell meiosis of the auto- and allotetraploid plants.

Table 4.18

Chromosome number of plants in six F_1 families

F_1 family Species	Code	Chromosome number observed	
L. rigidum	L3xB1	14	diploid
L. perenne	M1	14	
L. multiflorum	Lm4	28	
L. perenne	Lp2	28	tetraploid
L. perenne x *L. multiflorum* interspecific hybrid	PM1, PM2	28, 28	

5. DISCUSSION

5. DISCUSSION

5.1 F_1 families

In each F_1 family there was a complex pattern of cross-compatible, cross-incompatible and one way compatible reactions. Plants could not be arranged into groups which were both intra-incompatible and inter-compatible. In addition, all but one of the F_1 families had more than sixteen different mating types (there were only thirteen plants in family PM2: Fig. 4.13).

Variation in individual pollen tube penetration in compatible crosses indicated gametophytic control of pollen behaviour. This phenomenon was observed in each F_1 family studied. Sporophytic control of pollen behaviour would have resulted in a uniform pollen reaction. Gametophytic pollen determination has been found to be widespread in self-incompatible grasses and has previously been reported for L. perenne (Hayward and Wright, 1971; Spoor, 1976; Cornish et al., 1979a).

Self-compatible plants were observed in all F_1 families, but there was no difference in the overall proportion of self-compatible plants in diploid and tetraploid F_1 families (Appendix 1, Table 1). Thus, there was no evidence for a breakdown of the self-incompatibility system in tetraploid plants. The number of seeds set when flowering plants were bagged singly was low (maximum of twelve, family ED3: Table 4.2). If seed set had been used as the criterion of a compatible or incompatible reaction instead of pollen tube growth the numbers of seeds set would have been low enough for the reactions to have been classified as self-incompatible. As there did not appear to be any correlation between seed set in vivo and pollen

tube growth through self-stigmas *in vitro* it would appear that the self-incompatibility mechanism in *Lolium* species is weak and can be influenced by the environment. Variation in the efficiency of the self-incompatibility mechanism of *L. perenne* has previously been reported by Foster and Wright (1971) and Spoor (1976). Foster and Wright (1971) concluded that self-compatibility in *L. perenne* was the result of a genotype x environment interaction. However, there were no indications that "self-compatible" plants in this study had heterozygosity values of the same order or even the same sign (negative or positive) and in the thirteen F_1 families studied "self-compatible" plants did not appear to have similar S-locus genotypes. Thus, although self-compatibility was influenced by the environment, no genotype x environment interaction was demonstrated.

Incompatibility in the Gramineae has generally been shown to be controlled by two multiallelic loci, S and Z, with pollen behaviour determined gametophytically. The alleles of the two loci were shown to act independently in the style and an incompatible reaction only occurred when all the alleles present in the pollen grain were matched in the style (section 2.1.6.2). Therefore, a two locus system of this kind would result in a maximum of sixteen different mating types in an F_1 progeny when the parents had no alleles in common. A three locus control of incompatibility would result in 64 different mating types in an F_1 progeny if no alleles were shared by the parents and four locus control could produce 256 mating types in an F_1 progeny under similar conditions. Thus, the results obtained in thirteen F_1 families (Figs. 4.1 to 4.13) can be interpreted in terms of at least three loci controlling incompatibility in the

seven diploid F_1 families and perhaps six loci controlling incompatibility in the tetraploid F_1 families.

The conventional method for the analysis of data from diallel crosses has involved the number of mating types and the proportion of compatible and incompatible crosses. As the number of mating types observed indicated at least three loci controlling the incompatibility system, the observed and expected proportions of cross-compatible and cross-incompatible reactions in diploid F_1 families were compared (Table 4.3 of Table 5.1), by using χ^2 tests and the results are presented in Table 5.2. Because more than eighteen mating types were observed in all but one of the diploid F_1 families (ED3) and each family had a high proportion of cross-incompatible reactions, the observed results (Table 4.3) were compared to the theoretical values 96.2% + and 3.8% - (Table 5.1). As family ED3 (L. multiflorum) had only seventeen mating types the observed proportions of compatible and incompatible reactions (Table 4.3) were compared to the theoretical values 92.2% + and 7.8% -. The results of the χ^2 tests (Table 5.2) demonstrated that two of the diploid F_1 families, L3xB1 (L. rigidum, annual) and M1 (L. perenne, perennial) which displayed high frequencies of cross-compatible reactions could have been the result of crosses between parent plants sharing four alleles and having a three locus control of their incompatibility systems. However, as the plants used to produce these F_1 families were from different populations (L3xB1) or different experimental lines (M1) it seems unlikely that they would have had four alleles in common. It was interesting to note, in this context, that the proportion of cross-compatible

Table 5.1

Proportions of cross-compatible and cross-incompatible reactions expected in gametophytic self-incompatibility systems controlled by 1, 2 or 3 loci

No. of loci	No. of common alleles	% compatible pollen	No. mating types	+ (%)	- (%)
1	0	100	4	100	0
	1	50	2	100	0
2	0	100	16	100	0
	1	100	16	96.7	3.3
	2	75	12	93.9	6.1
	3	50	6	86.7	13.3
3	0	100	64	100	0
	1	100	64	100	0
	2	100	64	99.8	0.2
	3	87.5	56	98.4	1.6
	4	75	36	96.2	3.8
	5	50	18	92.2	7.8

Key: + cross-compatible reactions
- cross-incompatible reactions

NB: incompatible reaction occurs when all alleles in pollen and pistil are matched.

Table 5.2

Comparison of observed and expected proportions of cross-compatible and cross-incompatible reactions for each diploid F_1 family

Family	χ^2	probability
L. rigidum		
B5x08	58.31	<0.001
01x02	129.96	<0.001
L3xB1	0.70	0.50-0.30
L. multiflorum		
ED1	183.50	<0.001
ED2	196.48	<0.001
ED3	131.91	<0.001
L. perenne		
M1	2.80	0.10-0.05

reactions in family 01x02 was lower than in the two other L. rigidum F_1 families (B5x08 and L3xB1) (Table 4.3). The family 01x02 was the result of crossing plants from the same population, which might have had alleles in common. There were also low levels of cross-compatibility in the three L. multiflorum families which had been produced as a result of crossing plants from commercial varieties. This may indicate that some S-alleles have been unconsciously selected by plant breeders. This could have been brought about if the S-locus was linked to a desirable agronomic characteristic and many of the plants selected for this character had the same S-alleles.

A self-incompatibility system in which all the alleles in pollen and pistil, irrespective of the number of loci, have to be matched to produce an incompatible reaction cannot explain the occurrence of reciprocally incompatible plants, unless they have the same mating type. The occurrence in all the F_1 families of reciprocally incompatible plants which did not have the same mating types indicated that not all alleles present in pollen had to be matched in the pistil to cause an incompatible reaction. For example, the system proposed by Østerbye et al. (1980) can explain the phenomenon of plants with different genotypes being reciprocally incompatible.

In section 4.1 the proportions of paired reactions observed in the diploid F_1 families were compared using a χ^2 test, with those produced as a result of one parental combination of S and Z alleles (Table 4.4). Therefore, in order to extend this analysis, proportions of paired reactions were calculated based on one female and fourteen different male genotypes (Table 5.3). The theoretical

Table 5.3

Proportions of reciprocally compatible, reciprocally incompatible and one way compatible reactions expected when plants having 0 to 5 alleles in common are crossed

Gametophytic self-incompatibility system, where one locus has been duplicated (S'S''Z) and an incompatible reaction occurs when one S and one Z allele are matched (after Østerbye et al. (1980)).

	Female genotype: $S'_{12}S''_{34}Z_{ab}$								
	Male genotype			Number of common alleles	% compatible pollen	number of mating types	+⟷+ (%)	−⟷− (%)	+⟷− (%)
	S'	S''	Z						
i)	56	78	cd	0	100	64	90.5	9.5	0
ii)	16	78	cd	1	100	64	85.7	9.5	4.8
iii)	56	78	ac	1	100	64	79.4	9.5	11.1
iv)	16	37	cd	2	100	64	82.5	10.3	7.1
v)	12	78	cd	2	100	48	80.9	10.6	8.5
vi)	16	78	ac	2	75	48	72.7	11.3	16.0
vii)	16	37	ac	3	62.5	40	69.7	14.9	15.4
viii)	12	78	ac	3	50	24	60.9	21.7	17.4
ix)	16	78	ab	3	50	24	53.6	17.4	29.0
x)	12	37	ac	4	50	24	58.0	24.6	17.4
xi)	16	37	ab	4	25	12	45.4	36.4	18.2
xii)	12	78	ab	4	0	*			
xiii)	12	34	ac	5	50	18	55.6	28.8	15.7
xiv)	12	37	ab	5	0	*			

Key: +⟷+ reciprocally compatible
−⟷− reciprocally incompatible
+⟷− one way compatible
* plants are reciprocally incompatible but have different mating types.

genotypes had differing number of alleles in common which resulted in differing proportions of reciprocally compatible, reciprocally incompatible and one way compatible reactions in the progeny (Table 5.3). The expected and observed proportions of paired reactions in the seven diploid families were compared using χ^2 tests, taking into account the number of mating types and the proportion of reciprocally compatible crosses observed (Table 4.4 cf Table 5.3). The results of the χ^2 tests carried out are presented in Table 5.4 and show that in only one family, ED2 (L. multiflorum) did the observed proportions of the three types of reaction agree with expectations. Therefore, the F_1 family ED2 may have been the result of a cross between two plants which had three alleles in common ($S'_{12}S''_{34}Z_{ab}$ ♀ x $S'_{16}S''_{78}Z_{ab}$ ♂). The parents of ED2 were plants from commercial varieties of L. multiflorum, which due to inbreeding, may have resulted in a reduction in the number of alleles present in a population. The additional paired analysis which was made assuming reciprocal crosses to be identical (Table 4.5) was not used in comparisons with the theoretical proportions of paired reactions presented in Table 5.3. This was because each family displayed a different proportion of one way compatible reactions and this had not been allowed for when the paired analysis with reciprocals filled in was carried out. It is impossible to tell whether the proportions of one way compatible reactions observed (Table 4.4) were a true indication of the frequency of this type of reaction in the F_1 family as a whole.

In general, it appeared that the observed proportions of one way compatible reactions were too high compared to the expected proportions, and that the observed proportions of reciprocally

incompatible reactions were too low compared to expected proportions, thus resulting in the high χ^2 values obtained (Table 5.4). Therefore, this may indicate that certain combinations of alleles in the pollen grain may be able to overcome the self-incompatibility system and effect fertilisation when, in theory, they should be incompatible. The proportion of cross-incompatibility has been shown to be higher in a species where an incompatible reaction can occur when only some of the alleles present in the pollen are matched in the style (Tables 5.1 cf 5.3). Therefore, any factors which over-ride the incompatibility system would result in an increased frequency of cross-compatibility in the population.

In species having multilocus gametophytic self-incompatibility systems, the analysis of data produced by crossing full-sibs in diallel arrays has previously been based on the number of levels of one way compatibility observed. Therefore, this method of analysis appears to be unsuitable for use with the data obtained in this study since Larsen (1977a) and Østerbye (1975) postulated that all alleles must be matched in pollen and style to produce an incompatible reaction. In Ranunculis acris and Beta vulgaris the number of levels of one way compatibility present were equated with the number of heterozygous S-locci present (section 2.1.6.4). Therefore, certain genotypes, in accordance with their degree of S-locus heterozygosity may be ordered into a hierarchy where each member of one level is able to fertilise all members of lower levels, but only some, if any, at higher levels (Larsen, 1977a). Examples of plants which were one way compatible were observed in all the F_1 families, and, in addition, most F_1 families (except L3xB1, ED2, Lp1 and PM1) also contained examples of plants which showed a hierarchy of one way

Table 5.4

Comparison of observed and theoretical proportions of reciprocally compatible, reciprocally incompatible and one way compatible reactions, for each diploid F_1 family

Family	χ^2	probability
L. rigidum		
B5x08	10.80	0.01-0.001
01x02	15.58	<0.001
L3xB1	7.97	0.02-0.01
L. multiflorum		
ED1	11.60	0.01-0.001
ED2	1.13	0.70-0.50
ED3	6.34	0.05-0.02
L. perenne		
M1	6.67	0.05-0.02

NB: ED1 and ED3 cannot be compared to genotype xi (Table 5.3) as more than twelve different mating types were observed in these families.

Table 5.5

Examples of plants displaying expected and aberrant one way compatible reactions in each F_1 family

Species	Ploidy	Type of one way compatible reaction A ⟶ C	A ⟵ C	A ⟷ C	A.....C	Figure
L. rigidum	2x	Plants	Plants	Plants	Plants	
B5x08		2,16,12	28,27, 1	1,30,19	31,27,17	4.1
01x02		23, 8,15	22, 8,34	26,33,32	8,34, 4	4.2
L3xB1		-	-	7, 1,21	-	4.3
L. multiflorum						
ED1	2x	21,20,32	21, 5,22	27,32,22	34,32,14	4.4
ED2		-	-	17,39,29	-	4.5
ED3		14,31,24	14,31, 7	1,22,20	1,22, 6	4.6
Lm3	4x	6, 8, 5	6, 8,30	22,14,23	14,25, 5	4.7
Lm4		10, 5, 1	30, 5,31	8,12,23	31,17,20	4.8
L. perenne						
M1	2x	7,13, 5	7, 5,30	29,20,36	-	4.9
Lp1	4x	-	-	-	-	4.10
Lp2		28,11,14	7,11,14	14,22,37	13,37,27	4.11 (a) (b)
L. perenne x L. multiflorum interspecific hybrid	4x					
PM1		-	36,18,14	31,26,20	-	4.12
PM2		16, 5,24	-	5,24, 6	-	4.13

Key:

	A ⟶ C			A ⟵ C			A ⟷ C			A.....C		
	♂			♂			♂			♂		
	A	B	C	A	B	C	A	B	C	A	B	C
A	-	-	-	A	-	-	+	A	-	-	+	A	-	-	-
♀ B	+	-	-	♀B	+	-	-	♀B	+	-	-	♀B	+	-	-
C	+	+	-	C	-	+	-	C	+	+	-	C	-	+	-
	expected			aberrant								

- no examples of this type of reaction were found.

compatibilities (Table 5.5). Thus, plant A was able to fertilise plant B and plant C but plant B could only fertilise plant C and plant C could not fertilise either plant A or plant B.

		♂ A	B	C
	A	-	-	-
♀	B	+	-	-
	C	+	+	-

where + = compatible
and - = incompatible

The four F_1 families (Table 5.5) which did not include plants which displayed a hierarchy of one way compatible reactions included many blanks where crosses had not been performed. It is likely that if more pollinations had been performed that hierarchies of one way compatible plants would have been found. It was, therefore, interesting that during a study of the genetic control of self-incompatibility in tetraploid Secale cereale, Lundqvist (1957) postulated that if A, B and C were plants in the diallel and A was compatible as a pollinator on B and B was compatible as a pollinator on C, it followed that A would also be compatible as a pollinator on C. As A $\longrightarrow$ C (arrow indicates direction of compatible pollination) was the expected reaction the occurrence of any of the following:

(i) A $\longleftarrow$ C C compatible as pollinator of A;

(ii) A $\longleftrightarrow$ C reciprocally compatible;

(iii) A....C reciprocally incompatible would indicate that not all alleles have to be matched in pollen and pistil to produce an incompatible reaction. Observation of A $\longleftarrow$ C, A $\longleftrightarrow$ C and A....C reactions provided additional evidence that in tetraploid S. cereale

not all alleles in pollen and pistil had to be matched to produce an incompatible reaction. All the F_1 families (except Lp1 in which only one quarter of the possible crosses had been performed) contained examples of plants displaying aberrant one way compatible reactions (Table 5.5). Even the F_1 families which did not contain plants having a hierarchy of one way compatible reactions had plants which displayed aberrant one way compatible reaction types. Therefore, the observation of three levels of one way compatibility in conjunction with aberrant types of one way compatibility in both diploid and tetraploid F_1 families (Table 5.5) provides further evidence in support of the hypothesis that not all alleles in pollen and style have to be matched to produce an incompatible reaction in Lolium species.

One diploid F_1 family, B5x08 (L. rigidum) (Fig. 4.1) was examined in detail in order to estimate the frequency of expected and aberrant one way compatible reactions. In the A $\longrightarrow$ C (expected) reaction the plants A, B and C should have different numbers of heterozygous S-loci (Østerbye, 1975; Larsen, 1977a), and the assigned heterozygosity values were predicted to reflect this arrangement. Conversely, plants involved in aberrant one way compatible reactions could be expected to display non-linear heterozygosity values. The results obtained broadly agreed with expectations: 50% of the expected A $\longrightarrow$ C reactions produced plants which had linear heterozygosity values and 85.7% of the plants with aberrant reactions had non-linear heterozygosity values (Table 5.6). Overall, the ratio of expected : aberrant type reactions was 1 : 7. Conversely, chosing three plants at random with different heterozygosity values did not mean that they would display a hierarchy of one way compatibility.

Table 5.6

Frequency and examples of plants from family B5x08 (L. rigidum, diploid) having expected and aberrant one way compatible reactions, with corresponding heterozygosity values

Type of reaction	No. groups plants with linear H.V.		No. groups plants non-linear H.V.		total no. groups plants	
A ⟶ C (♂)	2		2		4	
A B C	Plant	H.V.	Plant	H.V.		
A - - -	2	-23.5	18	0		
♀ B + - -	16	- 7.0	2	-23.5		
C + + -	12	17.5	14	33.3		
A ⟵ C (♂)	0		2		2	
A B C			Plant	H.V.		
A - - +			28	1.8		
♀ B + - -			27	9.4		
C - + -			1	-15.0		
A ⟷ C (♂)	2		22		24	28
A B C	Plant	H.V.	Plant	H.V.		
A - - +	1	-15.0	16	- 7.0		
♀ B + - -	30	5.6	21	-12.3		
C + + -	19	8.3	13	10.4		
A.....C (♂)	2		0		2	
A B C	Plant	H.V.				
A - - -	31	- 6.2				
♀ B + - -	27	9.4				
C - + -	17	18.8				

Key: H.V. Heterozygosity Value

For example, plants 38, 11 and 13 (B5x08) had heterozygosity values of -24.9, 3.3‡ and 10.4 respectively and were all cross-compatible. Table 5.6 showed that the plants 31 and 17 (family B5x08) which were reciprocally incompatible had opposing heterozygosity values (-6.2 and 18.8 respectively), additional confirmation that they had different genotypes. Further investigation of all the reciprocally incompatible plants in family B5x08 (Table 5.7) again revealed differing heterozygosity values such that one plant had mainly heterozygous S-loci, the other mainly homozygous S-loci. The investigation of heterozygosity values of reciprocally incompatible plants was extended to cover all seven diploid F_1 families. Six possible classes for the pairing of heterozygosity values were considered: negative/negative, positive/positive, zero/zero, negative/positive, negative/zero and positive/zero (Table 5.8). The ratio of "like" to "unlike" pairings was 1 : 2.5 and the commonest individual type of pairing was negative/positive (41.1%: Table 5.8). However, despite "like" pairings of negative/negative, positive/positive and zero/zero none of the plants involved had identical genotypes.

When the plants from the F_1 family B5x08 (Fig. 4.1) were arranged in order of increasing heterozygosity value (most negative to most positive) (Fig. 4.15) there were six examples of the reaction heterozygous (het) ♂ x homozygous (hom) ♀ being incompatible, while the reciprocal cross (hom ♂ x het ♀) was compatible. It is difficult to explain this unexpected phenomenon using either a conventional multilocus system, in which all alleles must be matched to produce an incompatible reaction (Table 5.9) or on the novel system proposed by Østerbye et al. (1980) (Table 5.9). The theoretical

Table 5.7

Pairs of reciprocally incompatible plants from family B5x08 (*L. rigidum*, diploid) with corresponding heterozygosity values

Plants	Heterozygosity Value
8	20.8
2	-23.5
2	-23.5
30	5.6
11	3.3 ‡
1	-15.0
1	-15.0
13	10.4
3	- 1.3 ‡
21	-12.3
31	- 6.2
17	18.8

Key: ‡ Heterozygosity value expected to be zero, as plant was equally successful as a male and as a female.

Table 5.8

Frequency of six different types of Heterozygosity Value pairs for reciprocally incompatible plants in each diploid F_1 family

Family	Heterozygosity value pairing						total
	negative-negative	positive-positive	zero-zero	negative-positive	negative-zero	positive-zero	
L. rigidum							
B5x08				4	2		6
01x02	1	1		4	2	1	9
L3xB1					2		2
L. multiflorum							
ED1	4	1		9			14
ED2		3		1	1	2	7
ED3	3	1	1	4	3	2	14
L. perenne							
M1	1			1	2		4
Proportion of each type of pair	16.1%	10.7%	1.8%	41.1%	21.4%	8.9%	

Table 5.9

Possible explanations at the genotype level for results of crosses between plants having heterozygous and homozygous S-loci

Description of S-loci		Conventional system Sa,Sb,Sc (Lundqvist et al., 1973)		Novel system S'S''Z (Østerbye et al., 1980)	
		♀ genotype	♂ genotype	♀ genotype	♂ genotype
i)	♂ and ♀ heterozygous	Sa,Sb,Sc	Sa,Sb,Sc	S'S''Z	S'S''Z
	+ ↔ +	34,34,34	12,12,12	12,34,ab	56,78,cd
	- ↔ -	12,12,12	12,12,12	12,34,ab	12,78,ab
	+ ↔ -	*		*	
ii)	♂ and ♀ homozygous				
	+ ↔ +	11,11,12	33,33,34	11,34,ab	22,35,ab
	- ↔ -	11,11,12	11,11,12	11,37,ab	11,38,ab
	+ ↔ - (+	*		12,34,aa	11,34,ab
	+ ↔ - (-	*		11,34,ab	12,34,aa
iii)	♂ heterozygous ♀ homozygous				
	+ ↔ +	33,33,34	12,12,12	22,35,ab	12,34,ab
	- ↔ -	*		11,34,ab	12,34,ab
	+ ↔ - (+	11,11,12	12,12,12	12,34,aa	12,34,ab
	+ ↔ - (-	*		*	
iv)	♂ homozygous ♀ heterozygous				
	+ ↔ +	12,12,12	33,33,34	12,34,ab	22,35,ab
	- ↔ -	*		12,34,ab	11,34,ab
	+ ↔ - (+	*		*	
	+ ↔ - (-	12,12,12	11,11,12	12,34,ab	12,34,aa

Sa,Sb,Sc incompatible reaction: when all alleles in pollen and pistil are matched

S'S''Z incompatible reaction: when one S and one Z allele in pollen are matched in pistil

Key: * this type of reaction cannot be explained by the system under consideration.

crosses presented in Table 5.9 illustrate that plants having only heterozygous S-loci should not be one way compatible with each other. Of the plants in family B5x08 which had negative heterozygosity values, five were completely cross-compatible (Plants 1, 2, 7, 26, 38: Fig. 4.15). However, these plants displayed four examples of the het ♂ x hom ♀ incompatible (reciprocal cross being compatible) reactions. It is possible that these het ♂ x hom ♀ and hom ♂ x het ♀ crosses with unusual results were due to misclassification of the pollen reaction on the stigma. The technique used, of observing pollen tube growth by means of fluorescence gives a more accurate and immediate test of cross-compatibility or cross-incompatibility than seed set which may be influenced by many factors. Therefore, misclassification of pollen reactions seems unlikely. It is probably that the calculated heterozygosity value does not give an accurate reflection of the heterozygosity of the S-loci of a plant, especially where the male and female arrays are incomplete.

Despite the ability of the novel system proposed by Ósterbye et al. (1980) to provide an explanation for the phenomenon of reciprocally incompatible plants having different mating types it cannot explain one way compatibility at more than two levels. One way compatibility depends on homozygosity of S-loci but as incompatibility was postulated to occur when one S and one Z allele were matched in pollen and pistil the alleles of the S and Z loci cannot be homozygous in the same plant. Therefore, a family could, in theory, contain the genotypes $S'_{11}S''_{33}Z_{ac}$, $S'_{11}S''_{34}Z_{ac}$ and $S'_{16}S''_{37}Z_{aa}$ (parents: $S'_{12}S''_{34}Z_{ab}$ ♀ x $S'_{16}S''_{37}Z_{ac}$ ♂) but would not contain the genotypes $S'_{11}S''_{33}Z_{aa}$ or $S'_{11}S''_{37}Z_{aa}$ as the pollen grains $S'_{1}S''_{3}Z_{a}$ and $S'_{1}S''_{7}Z_{a}$ would be incompatible (one S and one Z allele

matched).

The results obtained in the diallel crosses of diploid L. rigidum, L. multiflorum and L. perenne indicate a multilocus (three or more) control of the incompatibility system, with gametophytic determination of pollen behaviour where not all alleles in pollen and pistil have to be matched to produce an incompatible reaction. One way compatibility can be explained by parent plants having alleles in common.

Two previous studies on self-incompatibility in L. perenne have produced similar results. Although Hayward and Wright (1971) did not observe more than sixteen mating types they did observe reciprocally incompatible plants having different mating types in three F_1 families studied. In contrast, Spoor (1976) observed 28 different mating types and a high proportion of reciprocal incompatibility (16%: Østerbye et al., 1980). In addition, the data presented by Hayward and Wright (1971) and Spoor (1976) showed hierarchies of one way compatible plants (three levels) and aberrant one way compatible hierarchies. Three locus control of the gametophytic incompatibility system has been postulated for Briza spicata (Murray, 1979) although two locus control has been demonstrated in B. media (Murray, 1974). An F_1 family of B. spicata had more than sixteen mating types, but in contrast to the Lolium results the proportion of cross-compatibility was high and plants which were cross-incompatible usually had the same mating types. Therefore, a multilocus system similar to Beta vulgaris and Ranunculis acris where S-loci cooperate to form specificities which must be matched to produce an incompatible reaction seems to be operating in B. spicata.

The genetic basis of self-incompatibility is usually similar in closely related groups but exceptions have been found in the

Solanaceae (Pandey, 1957; 1962a), Boraginaceae (Crowe, 1971; Varoupolos, 1979) and Caryophyllaceae (Lundqvist, 1979). Thus, Cornish et al. (1979a) considered that the genetic basis of self-incompatibility in L. perenne was likely to be the same as that demonstrated for Festuca pratensis to which it is related. Lundqvist (1955; 1961a) demonstrated a two-locus control of the self-incompatibility system of F. pratensis with gametophytic determination of pollen behaviour, in which all alleles present in the pollen grain had to be matched in the pistil to produce an incompatible reaction (section 2.1.6.2). In order to demonstrate two locus control of the incompatibility system the proportion of compatible pollen was assessed visually by Cornish et al. (1979a) into four classes: 0%, 50%, 75% and 100% compatible and in no instances were pollen grains counted. Other workers who have presented results as percentage pollen grains compatible have always counted the pollen grains and in doing so have observed less than the maximum percentage of compatible pollen grains expected in crosses between plants of known genotype (Hayman, 1956; Lundqvist, 1961a). For example, Hayman (1956) and Lundqvist (1961a) both observed less than 50% compatible pollen grains in crosses expected to be half compatible (Table 5.10) and less than 75% compatible grains in crosses expected to be three-quarters compatible (Table 5.10). In a cross expected to be fully compatible, Lundqvist observed twice as many compatible as incompatible pollen grains (Table 5.10). Therefore, relying on percentage compatible pollen could lead to confusion. In addition, if a gametophytic self-incompatibility system was controlled by three, not two, loci and all alleles had to be matched to produce an incompatible reaction the percentage compatible pollen could be 0%, 50%, 75%, 87.5% or 100%

Table 5.10

Ratios of compatible : incompatible pollen grains expected in theory, and observed in practice

Expected ratio	Observed ratio	Reference
+ : -	+ : -	
1 : 1	0.58-1.10 : 1	Hayman, 1956
	0.67 : 1	Lundqvist, 1961a
3 : 1	2.1 -2.5 : 1	Hayman, 1956
	1.3 : 1	Lundqvist, 1961a
fully compatible reaction	2.1 : 1	Lundqvist, 1961a

Key: + compatible
- incompatible

(Table 5.1). Alternatively, when only two alleles (one S and one Z) have to be matched to produce an incompatible reaction, as in the system proposed by Østerbye *et al.* (1980) the percentage compatible pollen could be 0%, 25%, 50%, 62.5%, 75% or 100% (Table 5.3). Cornish *et al.* (1979a) admit to misclassifying nine percent of the pollinations they observed, confusion arising between 50 and 75% compatible classes. Therefore, if either of the alternative systems proposed above controlled the incompatibility system in *L. perenne* it would be unlikely to be detected by visual assessment of the pollen reaction: if there was confusion between 50 and 75% there would be further confusion between 50, 62.5 and 75, or 75 and 87.5% compatible classes.

Weimarck (1968) studied self-incompatibility in the Gramineae and divided pollen reactions into 0%, 25%, 50%, 75% and 100% compatible classes. Significantly, a proportion of 25% compatible pollen cannot be explained by the two locus system proposed by Lundqvist (1954; 1955; 1956; 1961a) and Hayman (1956) but it can be explained by the system proposed by Østerbye *et al.* (1980).

In conclusion, the results presented by Cornish *et al.* (1979a) seem to point to a two locus control of the self-incompatibility system in *L. perenne* while the majority of the research on the genetic control of self-incompatibility in *L. perenne* indicates a multilocus control (Weimarck, 1968; Hayward and Wright, 1971; Spoor, 1976). This study fully supports the ideas presented by Spoor (1976) for a three locus control of the incompatibility system, not only in *L. perenne* but also in *L. rigidum* and *L. multiflorum*. Evidence presented in this study and by others (loc. cit.) indicates that not all the alleles in pollen and pistil have to be matched to produce an incompatible reaction but the presence of modifiers

may act to overcome the incompatibility system to ensure fertilisation when the level of cross-compatibility is low in a population (perhaps in a similar manner to the polygenic systems discovered in Borago officinalis (Crowe, 1971) and Myosotis scorpiodies (Varoupolos, 1979)).

It has been suggested (Lundqvist et al., 1973; Lundqvist, 1975) that inbreeding of experimental material, especially grasses, has led to the true number of S-loci present in a species being underestimated. Results obtained in this study and by Hayward and Wright (1971), Spoor (1976) and Murray (1979) indicate that the two locus self-incompatibility system is not universal within the Gramineae. Whether the multilocus system postulated for the genetical control of self-incompatibility in Lolium species will eventually be found to be universal within the Gramineae cannot be answered but must surely stimulate further investigation.

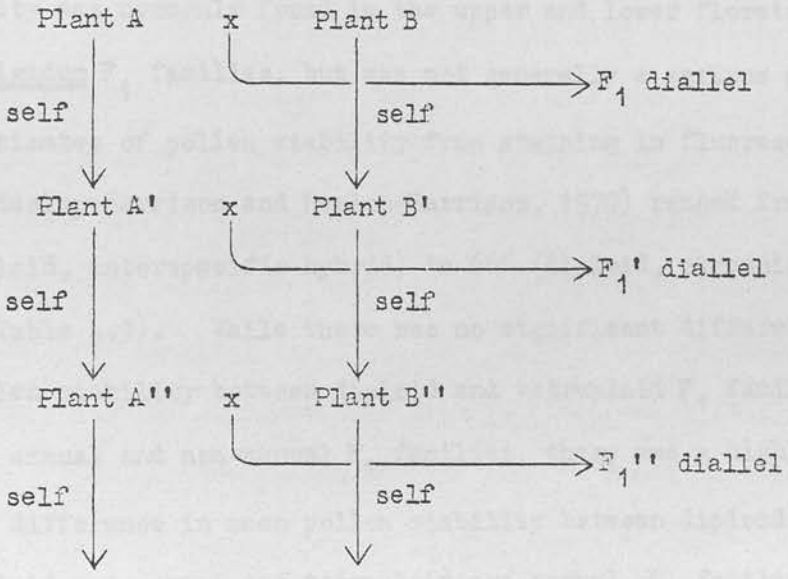
In order to investigate further the number of loci involved in the genetic control of self-incompatibility in Lolium species, selfing of plants in order to fix alleles is advocated. Although self-incompatible, some plants were observed to set seed when single flowering heads were bagged (Table 4.2). To start with two plants, A and B, would be crossed to produce an F_1 (which would be utilised in a diallel). At the same time the plants A and B would be selfed (Fig. 5.1). Any seed produced would be sown and two of the resulting plants (A', B') would then be crossed to produce a second F_1 (F_1'). The F_1' plants would then be crossed in a diallel array and the results compared to those from the F_1 diallel (Fig. 5.1). If any of the loci had become homozygous for the alleles carried in either of the plants A' or B', then the number of mating types in the F_1' diallel would be reduced when compared to the number in the F_1 diallel

(as the homozygous locus would not segregate). The possibility of selfing the plants A' and B' and producing fertile plants would be remote, but if achieved the resulting plants A'' and B'' and their progeny, F_1'', would provide valuable information if the number of mating types was further reduced in comparison with the F_1 and F_1' diallels (Fig. 5.1).

Although the petri dish technique (Lundqvist, 1961b) is a very efficient method of crossing plants, a means of storing Gramineae pollen without loss of viability would enhance <u>in vitro</u> pollination by reducing between plate variance within a single male array and ensuring pollen from early flowering plants was available for pollination of later stigmas.

Fig. 5.1

Suggested programme for further study of the genetical control of self-incompatibility in Lolium species



5.2 Pollen viability

Tests for pollen viability were used as a means of detecting male sterile plants and plants which produced a small amount of pollen, in order to exclude them from the diallel crosses. Plants which produced a limited quantity of pollen were not used as numerous flowering heads were required to collect sufficient pollen for each male array and this process tended to be wasteful of stigmas. Male sterility was commonly found in the upper and lower florets of the L. rigidum F_1 families, but was not generally a serious problem.

The estimates of pollen viability from staining in fluorescein diacetate (Heslop-Harrison and Heslop-Harrison, 1970) ranged from 51% (tetraploid, interspecific hybrid) to 66% (diploid, perennial F_1 family) (Table 4.9). While there was no significant difference in mean pollen viability between diploid and tetraploid F_1 families and between annual and non-annual F_1 families, there was a highly significant difference in mean pollen viability between diploid annual, diploid non-annual and tetraploid non-annual F_1 families (Table 4.10.i,ii,iii). In addition, there was a highly significant difference in mean pollen viability between different Lolium species (Table 4.10,iv). Manthiratna and Hayward (1973) also observed a difference in mean pollen viability between different Lolium species, when pollen grains were stained with nitroblue tetrazolium. However, Manthiratna and Hayward (1973) found that within the outbreeding species studied, L. multiflorum had a significantly higher pollen viability than L. perenne. In direct contrast, results obtained in this study indicated that L. perenne (both diploid and tetraploid F_1 families) had a higher mean pollen viability than L. multiflorum and an annual outbreeding species, L. rigidum (Tables 4.9, 4.10,iv).

The estimates of pollen viability obtained in the two separate studies for diploid L. perenne F_1 families by staining with fluorescein diacetate (65.77%: Table 4.9) and nitroblue tetrazolium (67.4%: Manthiratna and Hayward, 1973) were similar.

Pollen stainability could not be used as a basis to predict the amount of pollen tube growth in vitro because pollen from two F_1 families L3xB1 (diploid, annual) and PM2 (tetraploid, interspecific hybrid) did not always germinate and grow in vitro. For example, the mean pollen viability (estimated by staining with fluorescein diacetate) of the two L3xB1 plants which germinated and grew in vitro was 57.0%, and the mean pollen stainability of the nine plants which did not germinate and grow in vitro was 56.7%. Similarly, for the F_1 family PM2, the mean pollen stainability of plants which successfully germinated and grew in vitro was 51.0% and the mean pollen stainability of plants which did not germinate and grow in vitro was 53.7%. The measurements of pollen tube length for the four F_1 families (Table 4.11) in which all plants tested germinated in vitro were all made using the same culture medium. There have been indications that a medium which supports the highest percentage germination also allows the production of the longest tubes for Secale cereale (Pfahler, 1965) Zea mays (Pfahler, 1967) and Avena byzantina C. Koch (Wallace and Karbassi, 1968). Therefore, the medium used may not have resulted in maximum germination for the two perennial F_1 families M1 (diploid) and Lp2 (tetraploid), which produced tubes half the length of those produced by the F_1 families Lm4 and PM1 (Fig. 4.17). Thus, it would appear that pollen tube length may be related to the amount of energy expended during germination such that longer tubes were produced when germination required little energy. Tube lengths produced by culturing Zea mays

pollen grains *in vitro* have been shown to be dependent on the genotype of the plant producing the pollen grains (Pfahler and Linskens, 1973). This may indicate that adjustments in the culture medium would have resulted in longer tubes being produced when pollen grains from the perennial F_1 families M1 and Lp2 were cultured *in vitro*.

One of the F_1 families which produced pollen that did not germinate and grow successfully *in vitro* was an annual (*L. rigidum*). Although there have been previous reports of the successful *in vitro* germination and growth of pollen grains of *L. perenne* (Ahloowalia, 1973; Manthiratna and Hayward, 1973; Shivanna and Heslop-Harrison, 1978) and *L. multiflorum* (Ahloowalia, 1973; Manthiratna and Hayward, 1973), there have been no reports on the success or failure to culture *L. rigidum* pollen *in vitro*. It was reported by Manthiratna and Hayward (1973) that pollen from annual, inbreeding species (*L. remotum* and *L. temulentum*) did not germinate on media suitable for the *in vitro* culture of pollen from outbreeding *Lolium* species. This may indicate that the differences in pollen characteristics in relation to "*in vitro* germination" and growth are greater between annual and non-annual than between self-compatible and self-incompatible species.

The maximum tube lengths achieved by pollen of various grass species when cultured *in vitro* would not have been sufficient to effect fertilisation *in vivo* (Table 5.11). Unfortunately, Ahloowalia (1973) did not differentiate in the results presented between tube lengths produced by *L. perenne*, *L. multiflorum* and their interspecific hybrid. However, the maximum pollen tube lengths measured for haploid and diploid pollen cultured *in vitro* were greater in this study than

Table 5.11

Maximum tube length (μm) achieved by pollen of different grass species when cultured in vitro

Species	Maximum pollen tube length (μm)	Reference
Avena byzantina C. Koch	150	Wallace and Karbassi, 1968
Zea mays	405	Pfahler, 1965
Secale cereale	350	Shivanna and Heslop-Harrison, 1978
Pennisetum typhoideum	> 4,000	Vasil, 1960
Lolium (diploid spp.)	372	Ahloowalia, 1973
(tetraploid spp.)	420	
L. perenne (2x) M1	720	Plant 23
L. perenne (4x) Lp2	511	Plant 20
L. multiflorum (4x) Lm4	1,384	Plant 30
L. perenne x *L. multiflorum* (4x) interspecific hybrid PM1	1,350	Plant 23

those observed by Ahloowalia (1973) (Table 5.11). This difference may have been the result of the use of plants having different genctypes and the use of different culture media (liquid: Ahloowalia, 1973; semi-solid: this study). Although occasional tube lengths of more than 1,000 μm were recorded, these were rare; only one or two tubes for each plant grew to this length. Even the maximum tube lengths recorded *in vitro* would not have been sufficient to have achieved fertilisation *in vivo*. However, in view of the difference in conditions for germination between a culture medium and a stigma the maximum tube lengths achieved *in vitro* could not be expected to equal those required to effect fertilisation *in vivo*. In addition, any stimuli which, *in vivo*, enhance or encourage germination were unlikely to have been reproduced *in vitro*.

In conclusion, pollen stainability in fluorescein diacetate was found to provide a more rapid and reliable assessment of pollen viability than *in vitro* germination studies.

5.3 Anther and stigma length

Although there was no significant difference in mean anther size there was a highly significant difference in mean stigma size between the Lolium species studied (Tables 4.16.iv, and 4.17.iv). In general, families which had smaller anthers also had smaller stigmas (Table 5.12). In addition, the size of the anthers and stigmas appeared to be related to the size of the florets from which they were extracted. The largest anthers and stigmas were found in the tetraploid L. multiflorum F_1 family, Lm4, while the smallest anthers and stigmas came from the diploid F_1 families. The tetraploid interspecific hybrids had anthers which were smaller than the anthers of the two parental species, but the stigmas were of an intermediate size between those of L. perenne and L. multiflorum (Figs. 4.18 and 4.19).

Two of the F_1 families, M1 (L. perenne, diploid) and Lp2 (L. perenne, tetraploid) had mean anther lengths which were greater than the corresponding mean stigma lengths (Table 5.12). In addition, the F_1 families M1 and Lp2 displayed higher proportions of self-compatible plants than the families in which mean anther length was less than the corresponding mean stigma length (Appendix 1, Table 1; Table 5.2). Thus, a tendency towards self-pollination may have existed in the L. perenne F_1 families.

Table 5.12

Mean anther and stigma length (mm) for plants from six F_1 families

Family Species	Ploidy	Anther length (mm)	Stigma length (mm)
L. rigidum	2x		
L3xB1		3.46	3.49
L. perenne	2x		
M1		3.69	3.11
L. multiflorum	4x		
Lm4		4.00	4.67
L. perenne x *L. multiflorum* interspecific hybrid	4x		
PM1		3.82	4.47
PM2		3.80	3.89
L. perenne	4x		
Lp2		3.87	3.76

5.4 Cytology

The chromosome numbers of the plant material used in this study were found to be as stated by the supplier.

Despite observations of a low frequency of irregular pollen mother cell meioses in some auto- and allotetraploid plants, stainability of the pollen samples remained above 50%. In view of the large number of pollen grains shed by anemophilous plants only a much lower percentage of viable pollen grains than those observed (Table 4.9) would reduce the fertility of the Lolium species studied.

6. BIBLIOGRAPHY

6. BIBLIOGRAPHY

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APPENDIX 1

(Appendix 1)

Table 1

Results of pollinations for each F_1 family

Family Species	Ploidy	+	-	SI	SC	blanks	total	one way compatible (pairs)
L. rigidum	2x							
B5x08		346	78	20	5	176	625	41
01x02		372	127	19	6	101	625	90
L3xB1		557	32	24	3	113	729	19
L. multiflorum	2x							
ED1		325	137	18	8	138	676	70
ED2		197	87	25	2	418	729	16
ED3		116	73	14	3	83	289	27
Lm3	4x	314	164	17	7	74	576	91
Lm4		416	191	21	7	149	784	111
L. perenne M1	2x	466	35	11	12	5	529	27
Lp1	4x	106	37	21	4	457	625	6
Lp2		1107	139	27	11	160	1444	105
L. perenne x L. multiflorum interspecific hybrid	4x							
PM1		139	57	15	5	184	400	19
PM2		48	15	11	2	93	169	7

Key: + compatible
- incompatible
SI self-incompatible
SC self-compatible

blanks crosses not performed.

(Appendix 1)

Table 2

Paired pollination results for each F_1 family

Species	Ploidy	Family	+ ⟷ +	- ⟷ -	+ ⟷ -	blanks	total
L. rigidum	2x						
		B5x08	114	6	41	139	300
		01x02	117	9	90	84	300
		L3xB1	239	2	19	91	351
L. multiflorum	2x						
		ED1	79	14	70	162	325
		ED2	25	7	16	303	351
		ED3	29	14	27	66	136
	4x	Lm3	93	24	91	68	276
		Lm4	120	25	111	122	378
L. perenne	2x						
		M1	217	4	27	5	253
	4x	Lp1	14	4	6	276	300
		Lp2	441	11	105	146	703
L. perenne x L. multiflorum interspecific hybrid	4x						
		PM1	32	3	19	136	190
		PM2	9	3	7	59	78

Key: + ⟷ + reciprocally compatible
- ⟷ - reciprocally incompatible
+ ⟷ - one way compatible

blanks: crosses not performed including
blank ⟷ blank
blank ⟷ +
blank ⟷ -

(Appendix 1)

Table 3

Mean pollen viability (% grains stained in fluorescein diacetate) for plants from eight F_1 families

Plant	Family			
	01x02	L3xB1	M1	Lm3
1		66		
2		52		
3		44		*
4	*		78	
5		75	66	58
6	53	49		47
7		61	69	58
8	54	67		51
9		70		61
10	52	67	77	
11		53	61	54
12		59	70	
13	*	60	59	
14	65	57		56
15	47			
16		68	62	*
17		47	62	
18		65		69
19	58	73	69	57
20		59	62	
21	49	55		
22	59	57		*
23	47	58	77	69
24	42	46	57	
25	63	69	60	*
26	34	52	63	
27	64	68		49
28	*	62	63	
29	*	66	79	49
30	51		64	63
31	54			61
32	62		68	70
33	59		63	
34	67		60	
35	48		*	79
36	65		58	53
37	*			*
38				62
39				
40				51

Key: * missing value

01x02, L3xB1 } *L. rigidum*, diploid

M1 *L. perenne*, diploid

Lm3 *L. multiflorum*, tetraploid

(Appendix 1)

Table 3 contd.

Mean pollen viability (% grains stained in fluorescein diacetate) for plants from eight F_1 families

Plant	Family			
	Lm4	PM1	PM2	Lp2
1	67			59
2	*			65
3	44			57
4				57
5	52		49	59
6	73		49	60
7	57			67
8	71	49		65
9		54		68
10	60	49	53	69
11		50		68
12	66	52		65
13			52	50
14	51	53	*	63
15	65			67
16	41	51	52	*
17		52		70
18	*	59	63	65
19		54		69
20	64	53		69
21			72	68
22	*		*	65
23	*	56		*
24	61		51	
25	*	46		66
26	48	48		59
27		*	44	70
28		55	50	67
29	54		*	59
30	*			*
31	61	51		69
32	47			63
33	60	48		66
34		42		65
35	48			*
36	68	52		64
37				*
38	68			
39				*
40	59			*

Key: * missing value

Lm4 L. multiflorum, tetraploid

PM1, PM2 } L. perenne x L. multiflorum interspecific tetraploid hybrids

Lp2 L. perenne, tetraploid

(Appendix 1)

Table 4

Mean pollen grain diameter (μm) for plants from four F_1 families

Family			Pollen grain diameter (μm)		
Species	Ploidy	Plant	Mean	SEM	n
L. perenne	2x	4	31.01	0.88	66
M1		13	39.31	1.29	42
		20	32.58	1.26	36
		23	34.21	1.47	33
		28	31.89	1.14	44
		29	38.62	1.04	65
		30	35.84	1.19	56
		34	31.46	1.28	30
L. perenne	4x	4	43.21	2.33	17
Lp2		8	43.42	1.78	13
		13	42.40	1.72	30
		18	45.85	1.44	21
		20	44.20	1.25	41
		25	46.92	1.32	31
		30	44.70	1.42	24
		31	45.86	1.27	30
		33	44.51	1.46	29
		36	42.66	0.97	67
		39	45.35	1.28	22
L. multiflorum	4x	4	41.03	1.70	5
Lm4		8	49.68	2.48	23
		10	50.54	2.19	24
		16	36.65	3.65	9
		17	41.95	1.95	21
		18	41.44	2.54	11
		21	56.24	2.75	31
		24	40.56	2.11	17
		25	40.78	2.46	20
		30	43.50	1.73	18
		32	50.53	2.72	14
		37	47.59	2.37	20
L. perenne x	4x	12	42.27	3.54	9
L. multiflorum		16	47.22	4.56	9
interspecific		18	44.94	1.31	45
hybrid		19	48.50	2.26	21
PM1		20	43.09	1.97	15
		22	41.31	1.49	27
		23	41.94	1.45	30
		25	45.19	1.29	47
		28	44.53	1.49	21
		36	44.33	1.21	24

Key: SEM standard error of mean
n number of observations

(Appendix 1)

Table 5

Mean pollen tube length (μm) for plants from four F_1 families

Family Species	Ploidy	Plant	Pollen tube length (μm) Mean	SEM	n
L. perenne	2x	4	164.22	13.32	66
M1		13	92.52	8.24	42
		20	156.55	22.77	36
		23	222.57	32.85	33
		28	87.01	8.06	44
		29	114.13	10.97	65
		30	156.12	18.62	56
		34	84.52	7.87	30
L. perenne	4x	4	136.24	13.54	17
Lp2		8	153.71	28.07	13
		13	129.40	14.51	30
		18	78.59	10.51	21
		20	210.44	14.58	41
		25	100.61	9.55	31
		30	132.33	11.06	24
		31	91.49	6.84	30
		33	169.96	20.61	29
		36	98.78	6.09	67
		39	108.06	8.05	22
L. multiflorum	4x	4	236.86	53.91	5
Lm4		8	419.04	64.32	23
		10	363.11	52.10	24
		16	276.41	81.91	9
		17	370.39	58.77	21
		18	288.25	48.15	11
		21	407.07	22.24	31
		24	121.98	10.17	17
		25	336.86	55.99	20
		30	485.22	92.06	18
		32	252.26	53.72	14
		37	517.27	69.17	20
L. perenne x	4x	12	151.24	17.51	9
L. multiflorum		16	398.62	115.10	9
interspecific		18	408.06	44.28	45
hybrid		19	323.14	84.23	21
PM1		20	291.64	69.63	15
		22	286.84	34.38	27
		23	426.24	70.81	30
		25	379.70	37.05	47
		28	270.85	64.90	21
		36	561.66	73.08	24

Key: SEM standard error of mean
n number of observations

(Appendix 1)

Table 6

Mean anther length (mm) for plants from six F_1 families

Family Species	Ploidy	Plant	Anther length (mm) Mean	SEM	n
L. rigidum	2x	1	3.72	0.03	126
L3xB1		8	3.48	0.04	52
		12	3.88	0.04	58
		13	3.11	0.02	54
		18	3.65	0.07	51
		22	3.04	0.03	37
		25	3.39	0.02	63
		27	3.87	0.03	70
		28	3.04	0.02	95
L. perenne	2x	4	3.45	0.02	92
M1		10	4.19	0.03	79
		13	3.47	0.03	81
		20	3.44	0.03	83
		29	4.07	0.02	102
		30	4.01	0.03	47
		31	3.77	0.03	55
		34	3.31	0.03	62
		36	3.50	0.08	42
L. multiflorum	4x	24	3.98	0.02	65
Lm4		36	4.24	0.02	72
		38	3.79	0.04	43
L. perenne x	4x	18	4.43	0.02	53
L. multiflorum		19	3.48	0.03	35
interspecific		22	4.35	0.02	53
hybrid		23	3.86	0.02	54
PM1		26	3.27	0.03	22
		31	4.08	0.03	51
		33	3.59	0.03	29
		36	3.53	0.03	71
L. perenne x	4x	3	3.75	0.03	75
L. multiflorum		6	3.81	0.05	47
interspecific		7	4.02	0.02	93
hybrid		14	3.24	0.02	81
PM2		16	3.80	0.03	47
		18	4.43	0.07	48
		28	3.54	0.04	46
L. perenne	4x	13	3.71	0.03	51
Lp2		15	3.51	0.03	74
		25	4.24	0.02	63
		30	3.96	0.03	47
		33	3.86	0.05	68
		36	3.94	0.02	89
		39	3.85	0.02	93

Key: SEM standard error of mean

n number of observations

(Appendix 1)

Table 7

Mean stigma length (mm) for plants from six F_1 families

Family / Species	Ploidy	Plant	Stigma length (mm) Mean	SEM	n
L. rigidum	2x	1	3.17	0.05	48
L3xB1		8	2.98	0.11	23
		12	3.04	0.10	25
		13	3.37	0.11	27
		18	3.44	0.07	18
		22	4.17	0.16	18
		25	4.25	0.09	18
		27	3.08	0.08	29
		28	3.88	0.10	36
L. perenne	2x	4	2.98	0.05	37
M1		10	3.49	0.09	29
		13	2.57	0.08	29
		20	3.38	0.11	28
		29	3.13	0.06	36
		30	3.34	0.06	18
		31	2.91	0.08	20
		34	3.03	0.09	32
		36	3.16	0.09	17
L. multiflorum	4x	24	5.13	0.08	35
Lm4		36	4.63	0.09	44
		38	4.26	0.12	28
L. perenne x	4x	18	4.49	0.15	18
L. multiflorum		19	4.26	0.22	14
interspecific		22	4.85	0.10	30
hybrid		23	3.79	0.18	20
PM1		26	5.22	0.29	9
		31	4.20	0.15	19
		33	4.37	0.18	11
		36	4.60	0.11	41
L. perenne x	4x	3	4.40	0.11	35
L. multiflorum		6	3.88	0.09	23
interspecific		7	4.02	0.10	41
hybrid		14	3.68	0.15	28
PM2		16	3.21	0.05	17
		18	5.09	0.09	34
		28	2.95	0.12	20
L. perenne	4x	13	3.66	0.11	19
Lp2		15	3.33	0.08	26
		25	3.25	0.11	21
		30	4.43	0.17	18
		33	3.57	0.10	24
		36	4.20	0.07	31
		39	3.86	0.06	38

Key: SEM standard error of mean

n number of observations

APPENDIX 2

INCOMPATIBILITY IN LOLIUM MULTIFLORUM

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In the Gramineae, the majority of species investigated to date, (Briza media, Phalaris coerulescens, Secale cereale, Festuca pratensis, Hordeum bulbosum, Dactylis aschersonia and D. glomerata) exhibit a two locus (S and Z) multiallelic system with gametophytic control of pollen behaviour. An incompatible reaction occurs when both alleles present in the pollen are matched in the style. Interaction between the S and Z loci has not been demonstrated and it appears that the two loci have a physiological unity (Lundqvist, 1956).

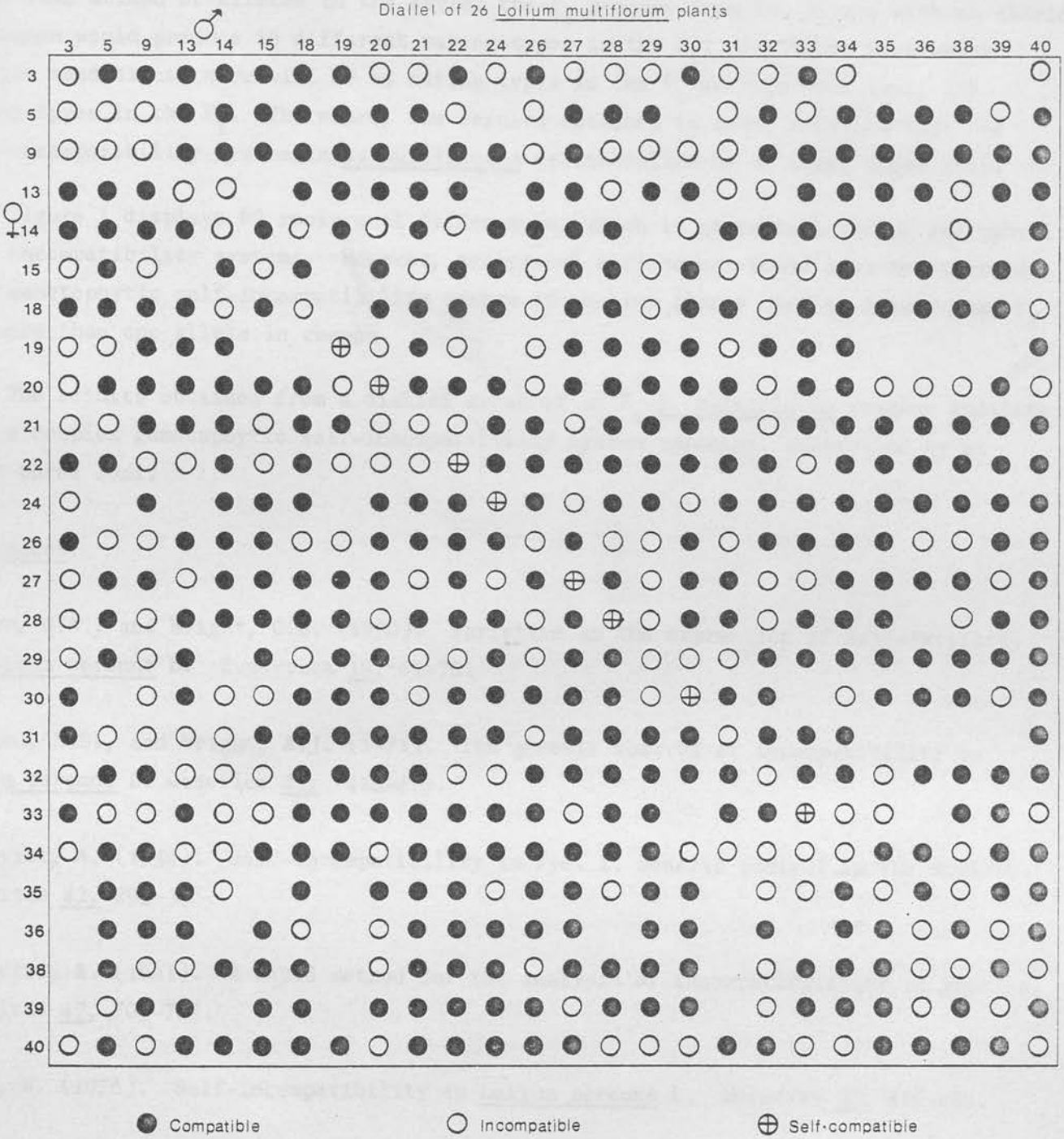
Lolium multiflorum was chosen in preference to L. perenne, as conflicting results (Weimarck, 1968; Hayman and Wright, 1971; Spoor, 1976) have been obtained in studies on the genetic control of self-incompatibility in L. perenne.

The technique used is based on the number of mating types present following a diallel cross of plants in an F_1 family. The petri dish technique of Lundqvist (1961) was used. Pollen tube growth in the stigmas was observed by staining whole pistils in a 0.2% solution of water soluble aniline blue in 0.1M k_3PO_4 for a few minutes, removing excess stain and mounting in glycerol (Dr. M.J. Lawrence, Birmingham - p.c.). A Leitz ortholux fluorescent microscope with BG12 and K515 filters was used to examine stained pistils.

The results of one diallel cross, using 26 L. multiflorum plants, are presented in Figure 1. Eight out of twenty-six plants used are self-compatible. To check these results all plants used in the diallel were selfed. Self-incompatible plants set no seed, whereas self-compatible plants set only a few seeds (maximum of 14 seeds). In cross-compatible combinations more than fifty seeds were usually produced. Variation in the expression of self-fertility with genotype has been observed in L. perenne by Foster and Wright (1971).

All 26 plants used in the diallel displayed different mating behaviour. Differential behaviour of pollen grains on stigmas indicated that a gametophytic self-incompatibility system was in operation. Assuming a two loci control of the incompatibility system,

Diallel of 26 Lolium multiflorum plants



both having multiple alleles and gametophytic determination of pollen behaviour and individual action of alleles in the style, the F_1 progeny from two plants with no alleles in common would produce 16 different mating types in the F_1. For three loci under similar conditions there will be 64 mating types in the F_1 and with four loci, 256 mating types in the F_1. Therefore, the results obtained to date, indicate that the self-incompatibility system in L. multiflorum is controlled by at least three loci.

Figure 1 displays 69 reciprocal differences, which is characteristic of sporophytic self incompatibility systems. However, reciprocal differences would also be expected in a gametophytic self-incompatibility system if the two plants used to produce the F_1 had more than one allele in common.

The results obtained from a diallel cross of an F_1 L. multiflorum progeny indicate that a complex gametophytic self-incompatibility system operates, controlled by at least three loci.

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